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Kenneth P. DuBois, Director

TABLE OF CONTENTS

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TABLE OF CONTENTS--Continued

THE EFFECTS OF IONIZING RADIATIONS ON THE BIOCHEMISTRY OF MAMMALIAN TISSUES

I. The Influence of X-irradiation on the Reductase Activity of the Livers of Rats

Kenneth P. DuBois and Bernard E. Hietbrink

This report concerns: The development of a quantitative method for measuring the reductase activity of mammalian tissues and application of the method to a study of the effects of x-irradiation on the activity of this enzyme in the livers of young rats.

Immediate or ultimate application of the results: The present investigation constitutes a continuation of systematic studies on the effects of ionizing radiations on the enzymatic reactions of mammalian tissues which have been in progress in this laboratory for several years. During the past few months our attention has been directed toward a study of individual steps in the hydrogen transport system as a result of the finding that the development of a detoxification system in the microsome fraction of the livers of young rats is inhibited by low doses of x-irradiation. The oxidative reactions concerned with drug metabolism in the liver microsomes require reduced triphosphopyridine nucleotide or diphosphopyridine nucleotide for their activity. The oxidation of chemicals by microsome enzymes takes place via a multi-step enzyme system. In an attempt to obtain information on the exact site of action of radiation, a study was undertaken on the individual steps in the hydrogen transport system. In some cases it has been necessary to develop assay methods applicable to the tissues of normal and irradiated animals to study the various steps of the overall reaction. The findings which have been made during this study have practical application with respect to the ability of irradiated animals to detoxify drugs and other foreign chemicals. It is anticipated that further studies on the exact site of action of radiation as an inhibitor of the development of microsome enzymes in young animals may contribute basic information on the biochemical effects of radiation which has not yet been obtained by studying the actions of ionizing radiations on tissues of adult animals.

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Evidence that sublethal doses of x-irradiation have a marked influence on the development of enzymes concerned with the metabolism of foreign chemicals by the liver was obtained in this laboratory several months ago (1). The initial evidence along this line came from measurements of the toxicity of a cholinergic organic phosphate to irradiated and normal young rats. In the normal animals the LD_{50} of the organic phosphate was 15 mgm./kgm. for 23-day old rats and at 45 days of age the value was 210 mgm./kgm. There was considerable evidence from a previous study by DuBois and Puchala (2) that the acquisition of resistance was due to the development of a detoxification system in the livers of young male rats. A direct approach was then undertaken to investigation of the possibility that irradiation inhibits the development of detoxification enzymes in the microsome fraction of the liver (3). The initial experiments (3) consisted of measurements of the rate of desulfuration of phosphorothioates. This oxidative reaction is catalyzed by a microsome oxidase and its development to the adult level occurs gradually during the first six weeks after birth of male rats (4). Rats exposed to 200 r or 400 r of radiation at the age of 23 days failed to exhibit the increase in enzyme activity to the adult level that normally occurs between the ages of 23 and 45 days (3). Evidence was obtained that this effect was not due to prolonged starvation or to a deficiency of reduced triphosphopyridine nucleotide. Although testosterone stimulates the development of the enzyme system (5) in the livers of male rats, shielding the testes during irradiation did not prevent the radiation-induced inhibition of development of the enzyme system.

Hietbrink et al. (6) recently employed partial body shielding to obtain information on the gross site of action of radiation in connection with the inhibition of the development of microsome oxidases. These experiments demonstrated that irradiation of only the liver area does not inhibit development of the enzyme system in contrast to the marked inhibition resulting from whole body irradiation. Since shielding the testes did not prevent the inhibition, it appears that tissues other than the liver and testes are able to supply a substance needed for the normal development of liver microsome enzyme systems. After whole body irradiation all sources of this unknown substance are apparently destroyed. This finding suggests the possibility that the findings made with respect to development of detoxification enzymes in the liver of irradiated animals may apply in some manner to other tissues. The substance which other tissues are apparently able to supply to the irradiated liver might also be involved in the synthesis of some enzyme system in the other tissues.

The findings described above stimulated our interest in further studies aimed at finding the exact site of the radiation-induced defect in the development of microsome enzymes. Since the catalytic action of these enzymes depends upon a source of reduced pyridine nucleotides generated by coenzyme-linked dehydrogenase systems, it seemed reasonable to initiate our studies on the individual steps of the reaction by ascertaining whether radiation inhibits the formation of reduced triphosphopyridine nucleotide. For this phase of the investigation the oxidation of glucose-6-phosphate and 6-phosphogluconic acid was studied (7). The dehydrogenases which catalyze the oxidation of both of these compounds require triphosphopyridine nucleotide and the reactions can serve as a source of the reduced coenzyme. Further interest was generated by a report (8) that lethal doses of radiation inhibit these dehydrogenases. Methods were developed using a tetrazolium dye as the hydrogen acceptor from reduced triphosphopyridine nucleotide thus eliminating the terminal steps involving the microsome oxidases and other systems capable of oxidizing triphosphopyridine nucleotide. The results of measurements of glucose-6-phosphate dehydrogenase activity demonstrated that 800 r of x-irradiation causes some inhibition of the enzyme activity but it did not exceed 40% in any tissue and was thus insufficient to account for the marked inhibition of the microsome enzyme systems. The 6-phosphogluconic acid dehydrogenase activity was likewise inhibited but to less than 30% in the livers of adult rats. Measurements were also made (9) of the effect of x-irradiation on the glucose-6-phosphate dehydrogenase activity of the livers of rats from 23 to 39 days of age. In contrast to the microsome oxidase activity, the activity of this enzyme reached the adult level by 25 days of age. Exposure of rats to 400 r had no inhibitory effect except to delay the increase in the enzyme activity to the normal adult level by about two days. It was clear from these experiments that x-irradiation does not inhibit pyridine nucleotide-linked dehydrogenases to a sufficient extent to account for the marked effect by radiation on microsome oxidase activity.

As a continuation of experiments directed toward attempts to elucidate the site of action of x-irradiation on the development of liver microsome enzymes, we have investigated the reductase activity of the livers of young normal and irradiated rats. The reductase system utilizes reduced triphosphopyridine nucleotide (10) and is present largely in the microsome fraction of the liver. The present report describes the development of a quantitative assay procedure for measuring the reductase activity of animal tissues and application of the method to the livers of normal and irradiated rats. The absence of an effect by radiation on the development of this enzyme system in young animals indicates specificity of the effect of radiation on the development of certain microsome enzymes.

Materials and Methods. Weanling and adult Sprague-Dawley rats were used for these experiments. The animals were kept in air-conditioned rooms and were fed Rockland Rat diet and water ad libitum.

X-irradiation was administered as single whole body exposures with a G. E. Maximar Therapy Unit. The radiation factors were 250 KVP, 15 ma., 0.25 mm. Cu and 1 mm. Al added filtration. The target-animal distance was 75 cm. and the dose rate was 35 r to 39 r per minute.

The measurement of reductase activity was made by the method developed during the course of this study. The details of the procedure and the experiments that were conducted to select the optimum conditions for the assay are described in this report.

Results

Development of a quantitative assay procedure for measuring the reductase activity of animal tissues. Fouts and Brodie (10) have studied the properties of a reductase system which catalyzes the conversion of nitro compounds to corresponding amines. They found that the enzyme system is present in both the soluble supernatant from which the nuclei, mitochondria and microsomes are removed and in the microsomes of liver. After dialysis the enzyme required the addition of reduced triphosphopyridine nucleotide (TPN) or a system capable of generating reduced TPN. Their initial studies were conducted on rabbit tissues and on the basis of experiments on tissues from this species they devised an assay system which was apparently considered to be quantitative since it was used to measure tissue distribution of the enzyme in rabbits and to study species differences in reductase activity of the liver. However, before using their method in our studies, it seemed desirable to ascertain whether it was a valid assay for enzyme activity particularly in view of the extremely high amounts of liver (0.5 and 1.0 gm.) and the high level of nicotinamide (100 micromoles) which they used. Their report (10) provides no evidence that experiments were conducted to determine the optimum concentration of each component of the reaction mixture.

In our initial experiments a system essentially the same as that of Fouts and Brodie (10) was used except that 50 mg. and 100 mg. of whole rat liver homogenate was employed and the reaction was carried out in a final volume of 3 ml. instead of 5 ml. P-nitrobenzoic acid was employed as the substrate. Its enzymatic reduction yields p-aminobenzoic acid which can be measured colorimetrically by the diazotization procedure of Bratton and Marshall (11). The test system used for our initial experiments contained 0.5 ml. of p-nitrobenzoic acid (1 mgm./ml.), 0.5 ml. of nicotinamide (10 mgm./ml.), 0.25 ml. of glucose-6-phosphate (20 mgm./ml.), 0.4 ml. of TPN (1 mgm./ml.), 0.5 ml. of 0.1 M phosphate buffer (pH 7.4), 0.5 ml. or 1.0 ml. of 10% whole liver homogenate and sufficient redistilled water to make a final volume of 3 ml. The constituents of the reaction mixture were placed in Warburg vessels and gassed with 95% nitrogen and 5% CO_2 for five minutes. After a 5-minute incubation equilibration period at 38° C., the liver homogenate was tipped from the side-arm into the main compartment of the vessel and the mixture was incubated for 60 minutes. At the end of the incubation period 2 ml. of 15% trichloracetic acid was added to each sample. The samples were transferred to centrifuge tubes and centrifuged for five minutes at 1,500 rpm. An aliquot of the reaction mixture (1.5 ml.) was then analyzed for free p-aminobenzoic acid. Under these conditions no free p-aminobenzoic acid was found in the reaction mixture when 50 mgm. of rat liver was used but when 100 mgm. or 200 mgm. of liver was employed, small amounts of p-aminobenzoic acid were present. However, a linear relationship was not obtained between the tissue level and the amount of p-nitrobenzoic acid which was reduced. It seemed possible that the lack of linearity might be due to acetylation of the p-aminobenzoic acid and that a higher percentage of the formed p-aminobenzoic acid was acetylated with the lowest tissue concentrations. Fouts and Brodie (10) reported that acetylation of p-aminobenzoic acid does not occur with rabbit liver homogenates and they assumed that it would not occur under the same conditions with liver homogenates from other species. As a consequence they reported extremely low reductase activity for rat liver and attributed the low activity to a species differences. However, we found that when the reaction mixture was subjected to acid hydrolysis by the addition of 0.3 ml. of concentrated hydrochloric acid followed by heating in a boiling water bath for 30 minutes, about 75% of the p-aminobenzoic acid had been acetylated. After hydrolysis the amount of p-aminobenzoic acid in the reaction mixture was strictly dependent upon the amount of tissue in the reaction mixture.

After completion of the experiments described above, it was possible to carry out additional tests to ascertain the optimum concentrations of the various components of the assay system. In this connection attention was first given to the optimum nicotinamide concentration because it seemed unlikely that a level as high as 100 micromoles was necessary to inhibit the breakdown of TPN. For this experiment 50 mgm. of homogenized rat liver was used in the test system described above and the nicotinamide concentration was varied from 0 to 5,000 μgm. At the end of the 60-minute reaction period 2 ml. of 15% trichloracetic acid was added. The reaction mixture was centrifuged and 1.5 ml. aliquots were used to measure free and total p-aminobenzoic acid. The results of this experiment are summarized in Table 1.

TABLE 1

INFLUENCE OF VARIOUS NICOTINAMIDE LEVELS ON THE REDUCTASE ACTIVITY OF RAT LIVER HOMOGENATES

Nicotinamide Concentration (µgm.)	Reductase Activity (µgm. of p-Aminobenzoic Acid Formed/100 mgm. of Liver/Hour)	
	Free	Total
0	7.0	34.6
100	6.8	34.4
250	6.6	34.0
500	6.6	32.0
1,000	6.6	28.4
2,000	6.0	25.2
5,000	2.0	17.0

The data in Table 1 show that it was not necessary to add any nicotinamide to a rat liver homogenate system containing 50 mgm. of liver and 400 µgm. of TPN. The activity was just as great in the absence of added nicotinamide as it was when 100, 250 or 500 µgm. were added. Of considerable significance, however, was the finding that high concentrations of nicotinamide exert a depressant effect on reductase activity. A level of 5,000 µgm. of nicotinamide, which is the amount used by Fouts and Brodie (10), depressed the enzyme activity by 50% as compared with the activity obtained with low levels or with no nicotinamide.

Further experiments were then conducted in which both the nicotinamide and the TPN levels were varied. In view of the higher activity obtained by decreasing the nicotinamide level to below that which inhibits the enzyme, it was possible to reduce the tissue level to 25 mgm. and 50 mgm. for the duplicate assays. The results of these measurements are summarized in Table 2. These data indicate that maximal activity can be obtained without the addition of either TPN or nicotinamide. Under the conditions of the assay there is, therefore, a sufficient quantity of endogenous TPN. The experiments also indicated that the activity was not decreased by the presence of various low levels of TPN and nicotinamide. In consideration of any possible applications of the assay procedure to situations in which there might be a deficiency of TPN, we selected a concentration of 100 µgm. of TPN and 100 µgm. of nicotinamide for the assay procedure to insure that TPN would not become the rate-limiting component of the system under any conditions that were anticipated.

TABLE 2

EFFECTS OF VARIOUS LEVELS OF TRIPHOSPHOPYRIDINE NUCLEOTIDE AND NICOTINAMIDE ON THE REDUCTASE ACTIVITY OF RAT LIVER HOMOGENATES

Triphosphopyridine Nucleotide Concentration (µgm.)	Nicotinamide Concentration (µgm.)	Reductase Activity (µgm. of p-Aminobenzoic Acid Formed/100 mgm. of Liver/Hour)	
		Free	Total
0	0	7.0	33.3
0	50	7.6	32.0
100	50	11.0	34.8
100	100	9.3	31.4
400	50	6.0	34.0
400	100	6.8	34.4

Additional experiments on the optimum conditions for the reductase assay were conducted in which various constituents of the reaction medium were omitted. The results of these measurements are summarized in Table 3.

TABLE 3

ESSENTIAL COMPONENTS OF THE REDUCTASE ASSAY SYSTEM FOR WHOLE RAT LIVER HOMOGENATES

Substance Omitted	Reductase Activity (µgm. of p-Aminobenzoic Acid/100 mgm./Hour)
None	32.4
p-Nitrobenzoic acid	0
Liver	0
Glucose-6-phosphate	32.8
Nicotinamide	33.4
TPN	33.0
TPN and nicotinamide	32.8

For these experiments the complete system contained 50 mgm. and 100 mgm. of liver, 5 mgm. of glucose-6-phosphate, 400 μgm. of TPN, 100 μgm. of nicotinamide and 0.5 mgm. of p-nitrobenzoic acid. The enzyme activity was expressed in terms of the total amount of p-aminobenzoic acid in the medium after acid hydrolysis. A reaction time of 60 minutes was employed. The data in Table 3 show that there is no p-aminobenzoic acid or other amines capable of giving the color reaction for p-aminobenzoic acid present in the reaction mixture when the substrate is omitted from the reaction mixture and there is no reduction of the substrate when liver is omitted from the test system. The activity was not affected when glucose-6-phosphate was omitted indicating that there is sufficient endogenous reduced TPN for the reaction. Similarly TPN and nicotinamide were not needed in the system. As a result of these measurements, it was evident that only the substrate and liver were essential when homogenates of normal, adult rat liver were assayed for reductase activity. However, glucose-6-phosphate, TPN and nicotinamide were added to the system in order to have better assurance that the procedure would not have to be altered for use on the tissues of immature and irradiated rats.

After establishment of most of the optimum conditions for measurement of reductase activity of rat liver, an additional experiment was performed to select the best tissue levels and incubation period. The experiments described above had indicated that amounts of liver within the range of 25 to 100 mgm. were satisfactory with a 1-hour incubation period. To obtain further information on these points a comparison of the enzyme activity was made using 25, 50 and 100 mgm. of whole liver homogenate from male rats with incubation periods of 30 and 60 minutes. The results of this experiment are summarized in Table 4. The results are expressed in terms of the total amount of p-aminobenzoic acid in the medium under each experimental condition and as the amount per 100 mgm. of tissue per 60 minutes. The results indicate that the activity is dependent upon the tissue level and the incubation time. The only deviation from linearity occurred with 100 mgm. of liver and a 60-minute incubation period. On the basis of this experiment we selected a 60-minute reaction time for all subsequent experiments. Two tissue levels of 25 and 50 mgm. were used for each assay on the livers of adult animals in subsequent experiments. When the activity was lower as it was in the livers of weanling rats, 50 and 100 mgm. of liver were used and during the age period when the reductase activity of the liver was increasing, three levels of tissue (25, 50 and 100 mgm.) were used.

On the basis of the experiments described above, the final assay system developed for measuring the reductase activity of animal tissues contained the following constituents: 0.5 ml. of p-nitrobenzoic acid (1 mgm./ml.), 0.25 ml. of glucose-6-phosphate (20 mgm./ml.), 0.1 ml. of triphosphopyridine nucleotide (1 mgm./ml.), 0.1 ml. of nicotinamide (1 mgm./ml.), 0.5 ml. of 0.1 M phosphate buffer (pH 7.4), 0.25 and 0.5 ml. of 10% whole liver homogenate (25 mgm. and 50 mgm.), and sufficient distilled water to make a final volume of 3.0 ml. The reaction mixture was placed in Warburg vessels with the liver in the side-arms. The vessels were gassed with 95% nitrogen and 5% carbon dioxide for five minutes. After five minutes equilibration at 38° C., the liver was tipped from the side-arms into the main compartments of the vessels and the reaction was carried out for one hour. At the end of this period 2 ml. of 15% trichloracetic acid was added. The samples were placed in centrifuge tubes and centrifuged for five minutes at 1,500 rpm. One aliquot (1.5 ml.) of the reaction mixture was used for measurement of the amount of free p-aminobenzoic acid in the medium and another 1.5 ml. aliquot was hydrolyzed by addition of 0.3 ml. of concentrated hydrochloric acid with heating in a boiling water bath for 30 minutes. The total p-aminobenzoic acid was measured on this sample.

TABLE 4

VARIATION IN REACTION TIME AND TISSUE LEVEL ON THE REDUCTASE ACTIVITY OF THE LIVERS OF ADULT, MALE RATS

Liver (mgm.)	Reaction Time (Minutes)	Reductase Activity	
		μgm. p-Aminobenzoic Acid Per Sample	μgm. p-Aminobenzoic Acid/100 mg./Hour
25	30	5.6	44.8
50		10.6	42.6
100		22.3	44.6
25	60	11.1	44.4
50		22.6	45.2
100		34.3	34.3

<u>Reductase activity of the livers of adult rats and mice.</u> The reductase assay procedure described above was first applied to measurements of the enzyme activity of the livers of adult male and female rats and to the livers of male mice. For these measurements groups each containing four animals were used. The assays were performed in duplicate using 25 mgm. and 50 mgm. of whole liver homogenate. Both the free p-aminobenzoic acid and the total amount after hydrolysis of the acetylated amine were measured. The latter value gives the true indication of reductase activity and the difference between the total and free p-aminobenzoic acid represents the amount conjugated presumably by acetylation. The results of these measurements are summarized in Table 5 where the average and range of values are presented. It may be seen from these data that the individual differences in enzyme activity were small. There was no sex difference in the activity of the enzyme in rat liver. This finding may be contrasted with the 2 to 3-fold higher activity of certain oxidative enzymes in the liver microsomes of male rats than in females (5).

Other investigators have reported (10) that the reductase activity of rat liver is extremely low. They obtained only 0.17 μM of p-aminobenzoic acid from incubation of p-nitrobenzoic acid with 500 mgm. of liver for three hours. When our results are calculated on the same basis, we obtain 3.1 µM of p-aminobenzoic acid which is 18 times the activity reported by Fouts and Brodie (10). These investigators also reported that mouse liver has about nine times higher activity than rat liver in contrast to the absence of a species difference in our experiments. Since they did not take into consideration the acetylation of p-aminobenzoic acid or the depressant action of high levels of nicotinamide, the validity of their conclusions is doubtful.

TABLE 5

REDUCTASE ACTIVITY OF THE LIVERS OF ADULT RATS AND MICE

Species	Sex	Reductase Activity (µgm. p-Aminobenzoic Acid/100 mgm./Hour)			
		Free		Total	
		Average	Range	Average	Range
Rat	Males	8.2	(7.5-9.3)	28.7	(27.0-30.0)
Rat	Females	9.6	(7.8-10.7)	29.6	(28.6-30.6)
Mice	Males	11.6	(10.7-12.7)	28.4	(25.9-29.9)

Rate of development of reductase activity in the livers of young male rats. To ascertain whether the reductase activity of the liver of young rats increases during the period prior to six weeks of age, assays were conducted at intervals from 22 to 42 days of age. The results of these measurements are summarized in Table 6 where the average and range for groups of four animals are presented. The results are also shown graphically in Figure 1. These assays demonstrated that the reductase activity of the liver is less than one-half the normal adult level at 22 days of age. The activity increases at a relatively rapid rate and reaches the normal adult level by 35 days of age.

Influence of 400 r of whole body x-irradiation on the development of reductase activity in the livers of young male rats. The influence of x-irradiation on the development of reductase activity in the livers of young male rats was measured on three groups each containing four young rats. All of the animals were irradiated at 23 days of age when the reductase activity was about one-half of the normal adult level. One group of animals was sacrificed at 31 days of age, another group was sacrificed

TABLE 6

RATE OF DEVELOPMENT OF REDUCTASE ACTIVITY IN THE LIVERS OF YOUNG MALE RATS

Age (Days)	Reductase Activity (µgm. p-Aminobenzoic Acid/100 mgm./Hour)			
	Free		Total	
	Average	Range	Average	Range
22	4.5	(3.2-5.5)	14.6	(13.8-15.5)
26	6.7	(6.2-7.2)	19.7	(18.9-20.4)
30	8.8	(8.2-9.6)	26.4	(23.6-28.4)
35	11.5	(11.1-12.0)	35.1	(33.2-36.5)
42	8.9	(8.1-9.3)	30.5	(29.0-32.0)

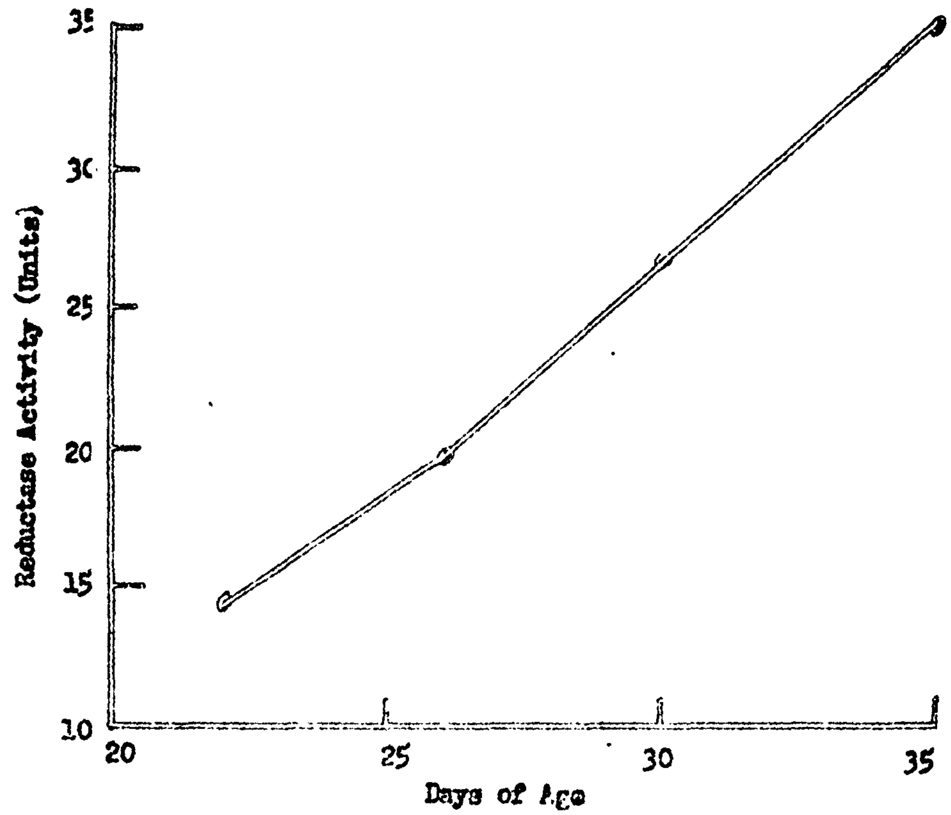

Figure 1. Rate of development of reductase activity in the livers of young male rats.

at 35 days of age and the third group was sacrificed at 44 days of age. The average and range of values for the reductase activity of the livers of these animals is shown in Table 7.

TABLE 7

INFLUENCE OF 400 r OF X-RAY ON THE DEVELOPMENT OF REDUCTASE ACTIVITY IN THE LIVERS OF YOUNG, MALE RATS

Age at Time of X-ray (Days)	Age at Time of Sacrifice (Days)	Time of Sacrifice After X-ray (Days)	Reductase Activity (μgm. of p-Aminobenzoic Acid/100 mgm. Tissue/Hour	
			Free	Total
23	31	8	10.4 (10.0-11.0)	27.2 (25.6-28.0)
23	35	12	10.8 (10.1-11.4)	30.8 (28.6-32.5)
23	44	21	11.3 (11.0-11.7)	32.0 (30.3-34.4)

A comparison of the values shown in Table 7 with those presented in Table 6 indicates that the rate of development of the enzyme activity to the normal level was not affected by x-irradiation. Thus it may be concluded that the marked inhibitory effect of irradiation on the development of microsome oxidase systems (3,6) represents a selective effect of x-irradiation.

Discussion

The present investigation was undertaken to extend knowledge of the effects of ionizing radiations on the individual steps of the hydrogen transport system of animal tissues. In the present study the enzymatic reduction of nitro compounds to amines was studied. This reaction is catalyzed by a flavoprotein reductase enzyme system located partly in the microsome fraction of the liver and it requires reduced triphosphopyridine nucleotide for activity. Previous studies in this laboratory (3,6) have demonstrated that low doses of radiation markedly inhibit certain oxidative reactions catalyzed by microsome enzymes in the livers of young male rats. It was, therefore, of considerable interest to ascertain whether radiation inhibits the development of other microsome enzymes.

In order to conduct the present study, it was necessary to develop a quantitative assay procedure for measuring the enzymatic reduction of foreign chemicals by the liver. Fouts and Brodie (10) have reported some studies on this enzyme but investigation of their assay system indicated that it does not meet the criteria of a valid quantitative enzyme assay procedure. A study of the optimum conditions for measurement of the reductase activity of animal tissues resulted in the development of a quantitative procedure in which the rate of the reaction was strictly dependent upon the tissue level. Under the conditions of the assay the reductase activity of the livers of adult rats was 18 times higher than it was in the system used by Fouts and Brodie (10) and we found no species difference in the reductase activity of rat and mouse liver.

Application of the method to measurement of the reductase activity of the livers of young male rats indicated that the activity at 22 days of age is about 40% of the adult activity. Exposure of 23-day old rats to 400 r did not inhibit the rate of development of reductase activity of the liver. This finding indicates that x-irradiation has a selective action in its inhibitory effect on the development of certain enzymatic reactions catalyzed by microsome enzymes.

The present study as well as other experiments in this laboratory (7,9) on the hydrogen transport system have demonstrated that radiation does not affect reactions which generate the reduced triphosphopyridine nucleotide needed for oxidation and reduction reactions catalyzed by microsome enzymes. Our experiments to date have provided a considerable amount of evidence that radiation exerts its inhibitory action on a reaction between reduced triphosphopyridine nucleotide and the oxidizable substrate. Additional studies are in progress in an attempt to locate the exact site of the radiation-induced defect in the development of certain microsome enzymes in the liver.

Summary

1. A study was undertaken of the influence of x-irradiation on the reductase activity of animal tissues. For this study the enzymatic reduction of p-nitrobenzoic acid to p-aminobenzoic acid was used as a measure of reductase activity. A quantitative method for measuring the reductase activity of animal tissues was developed by the conduction of a series of experiments to determine the optimum conditions for the reaction.

2. The assay procedure developed during this study was applied to the tissues of normal adult male and female rats and normal adult mice. No sex difference was observed in the enzyme activity of the livers of rats and no species differences were observed in a comparison of male rats and male mice.

3. Application of the assay procedure for measurement of the reductase activity of the livers of young male rats indicated that the enzyme activity is less than half the adult level at 22 days of age and it increases to the adult level by 35 days of age.

4. Exposure of 23-day old male rats to 400 r of whole body x-irradiation had no effect on the rate of development of the reductase activity of the livers in contrast to the marked inhibitory effect of this dose of radiation on the development of those microsome enzymes in the liver which catalyze oxidative changes in foreign chemicals.

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THE EFFECTS OF IONIZING RADIATIONS ON THE BIOCHEMISTRY OF MAMMALIAN TISSUES

II. Influence of X-irradiation and a Nitrogen Mustard on the Development of a Thiophosphate-Oxidizing Enzyme System in the Livers of Young Male Rats

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Kenneth P. DuBois

This report concerns: The results of additional experiments in a study recently undertaken to obtain information on the effect of ionizing radiations on the development of the phosphorothioate-oxidizing enzyme system in the livers of young male rats. The present study was primarily concerned with the ability of partial body shielding and 2-mercaptoethylamine (MEA) to reduce the degree of radiation-induced inhibition of the synthesis of the enzyme system. Experiments were undertaken to obtain information on the influence of the radiomimetic agent, methyl bis(2-chloroethyl)amine (HN2), on the development of this detoxification mechanism. The effect of MEA on the radiation-induced inhibition in the normal development of resistance in young male rats to the cholinergic phosphorothioate, 0,0-diethyl 0-(4-methylthio-m-tolyl) phosphorothioate (DMP), was also tested.

Immediate or ultimate application of the results: Results of our recent studies have shown that ionizing radiations inhibit the development of the enzyme system responsible for the oxidative desulfuration of certain drugs and toxic agents in the livers of young rats. Initial studies indicated that doses of x-irradiation as low as 100 r markedly reduce the rate of development of this detoxification mechanism. In subsequent experiments it was found that the radiation-induced inhibition of the development of this enzyme system is reversible at four to six weeks after 100 r or 200 r of x-ray. It was also found that when the liver area is exposed to 400 r of x-irradiation with the remainder of the body shielded, inhibition of the synthesis of these enzymes does not occur, and that the inhibition caused by 200 r of radiation can be prevented by the administration of MEA prior to x-ray exposure. The present investigation is a continuation of these studies and it has been primarily concerned with the effect of higher doses of radiation on the development of the microsome oxidases in the livers of partially shielded or MEA-treated rats. The results of these studies indicate that the administration of 600 r of x-irradiation to the liver area causes a substantial inhibition in the development of this enzyme system and that MEA prevents the marked inhibitory effect of 400 r of total-body x-ray. It is anticipated that experiments of this type will provide further information which will lead to a more complete understanding of the influence of ionizing radiations on the biochemical consituents of animal tissues.

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During the past several months we have been investigating the effects of ionizing radiations on the synthesis of enzymes in the livers of young male rats which catalyze the metabolism of dimethyl-2-(4-oxo-1,2,3-benzotriazinyl-3-methyl) phosphorodithioate (guthion, DBD). Previous studies in this laboratory (1) showed that x-irradiation markedly inhibits the synthesis in regenerating liver of the enzymes which catalyze the oxidative desulfuration of this phosphorodithioate but that radiation had no effect on the microsomal enzyme activity in the livers of adult rats. These findings indicated that x-ray inhibits the formation of the enzyme system but does not affect the activity of existing enzymes. Studies were then undertaken to obtain information concerning the influence of ionizing radiations on the synthesis of the enzyme system in the livers of young rats which is responsible for the oxidative desulfuration of certain chemical agents.

The initial studies on the effects of radiation on the synthesis of the phosphorothioate oxidizing enzyme systems in the livers of young male rats demonstrated that 200 r and 400 r of x-ray almost completely inhibit the normal development of the enzyme system during the first three weeks after exposure (2). Subsequently it was found that doses of total-body x-irradiation as low as 100 r caused substantial reductions in the rate of synthesis of the enzyme system (3) and that this inhibition is reversible at four to five weeks after 100 r or 200 r of x-ray (4). Experiments undertaken to determine whether a radiation-induced decrease in androgens was responsible for the delay in development of this enzyme showed that shielding of the testes or daily injection of testosterone propionate did not prevent the inhibitory effect of 200 r or 400 r of x-irradiation on the synthesis of the drug metabolizing enzyme (3). In view of these results, it was of interest to determine the effect of shielding the body while irradiating the liver area. It was found that the administration of 400 r of x-irradiation to the liver area only does not inhibit the development of the enzyme responsible for drug metabolism in this tissue. The present report describes the results of additional experiments on the influence of higher doses of x-ray to the liver area on the synthesis of this enzyme system.

Experiments were undertaken to obtain information concerning the influence of MEA, one of the most effective radioprotective agents in rats, on the radiation induced inhibition of the development of the phosphorothioate-oxidizing enzymes of the liver. It was found that 200 mgm./kgm. of MEA given ten minutes before 200 r of x-irradiation completely prevented the inhibition of enzyme synthesis caused by this dose of radiation (4). The results of further studies concerning the ability of MEA to prevent the radiation-induced inhibition of the synthesis of the liver oxidase system are described in this report. Data are also included in the present report on the effect of the radiomimetic compound, HN2, on the synthesis of this enzyme system and the influence of MEA on the development of resistance in x-irradiated, young male rats to the toxicity of O,O-diethyl O-(4-methylthio-m-tolyl) phosphorothioate (DMP).

Materials and Methods. Young, male Sprague-Dawley rats were used for these experiments. The animals were maintained in air-conditioned quarters and were given Rockland Rat Diet and water ad libitum. X-irradiation was administered as a single exposure with a G. E. Maximar therapy unit employing the following radiation factors: 250 KVP, 15 ma., 0.25 mm Cu and

1 mm. Al added filtration. The target-animal distance was 75 cm. giving a dose rate of 34 r to 36 r per minute as measured in air with a Victoreen ionization chamber. For experiments on the effect of partial body shielding on the development of the drug metabolising enzyme system, weanling rats were anesthetised with aqueous solutions of sodium pentobarbital (25 mgm./kgm. intraperitoneally) to facilitate accurate placement and maintenance of the lead shields during radiation exposure. Aqueous solutions of HN2 and neutral aqueous solutions of MEA were prepared daily and injected intraperitoneally within 15 minutes in all instances.

For the enzyme assays the rats were sacrificed by decapitation and the livers were quickly removed, weighed and homogenised in cold distilled water. Solutions of guthion (0.1 M) were prepared in warm ethanol. The alcoholic solution was then diluted to 1×10^{-4} M with distilled water. Guthion was converted to its active metabolite by the method developed by Murphy and DuBois (5) in this laboratory and by a modification of the method used by Conney et al. (6) for other reactions catalysed by microsome enzymes. The latter method utilises 0.1 ml. of triphosphopyridine nucleotide (TPN) (1 mgm./ml.), 0.4 ml. of glucose-6-phosphate (10 mgm./ml.), 0.4 ml. of adenosine triphosphate (ATP) (1×10^{-2} M), and 0.1 ml. of potassium chloride (2 M) in addition to 0.4 ml. of 0.1 M phosphate buffer (pH 7.2), 0.3 ml. of nicotinamide (1×10^{-3} M) and 0.5 ml. of diphosphopyridine nucleotide (DPN) (1 mgm./ml.). Each Warburg vessel also contained 0.1 ml. or 0.2 ml. of a 2.5% aqueous homogenate of liver (2.5 mgm. or 5 mgm. of tissue), 0.3 ml. of aqueous guthion solution and sufficient water to make a final volume of 3.0 ml. These mixtures were incubated for ten minutes at 38° C. following an initial 5-minute equilibration period. A 0.6 ml. aliquot of the reaction mixture was added to the cholinesterase test system of DuBois and Mangun (7) and the amount of active metabolite formed was determined from the amount of inhibition of rat brain cholinesterase activity. The activity of the enzymes which catalyze the oxidation of guthion was expressed in terms of arbitrary units of active metabolite formed per 5 mgm. of fresh liver per hour and was calculated according to the procedure of DuBois et al. (8). Qualitatively similar effects were observed in both systems but the system containing the reduced triphosphopyridine nucleotide was about twice as active. The data presented in this report was obtained using the more active system.

Results

Influence of partial body shielding on the development of the phosphorothioate-oxidising enzyme system in the livers of young male rats. The results of previous studies have shown that shielding the testes does not influence the inhibitory effect of 200 r of x-ray on the development of the phosphorothioate-oxidising enzyme system in the liver (2). More recent experiments (3) have illustrated that this inhibitory effect of 200 r of x-irradiation on the enzyme synthesis was substantially reduced by shielding the liver area and that when the liver area was exposed to 200 r or 400 r of x-ray with the remainder of the body shielded, development of the drug metabolising enzyme system in the liver was not inhibited. The present study was undertaken to determine the influence of higher doses of x-irradiation on the development of the enzyme system in the liver. For these experiments 23-day old male rats were anesthetized with 25 mgm./kgm. of sodium pentobarbital and lead shields were placed so as to shield the entire body except the liver area. This area was then given 600 r of x-irradiation. The animals were sacrificed at various intervals during the following three weeks, a portion of the liver was removed and the microsome oxidase activity was measured. The results of these measurements are shown in Figure 1 where each point on the curves is the average of measurements on the livers of at least three animals.

The effect of 200 r of whole body x-ray and of 400 r of radiation to the liver area on the development of the enzyme system in the livers of young rats has been presented in previous reports and is included in Figure 1 for purposes of comparison. The data illustrate that 600 r of x-ray to the liver area, like 400 r to this area, enhances the development of the phosphorothioate oxidizing enzymes in the liver for approximately 10 to 12 days following the radiation exposure, however, there was a substantial inhibition in the rate of synthesis during the 12 to 21-day period following 600 r of x-irradiation.

Influence of 2-mercaptoethylamine on the radiation-induced inhibition of the development of the phosphorothioate-oxidizing enzyme system in the livers of young male rats. The results of studies presented in our previous report (4) showed that 200 mgm./kgm. of MEA prevented the inhibition of the development of the phosphorothioate-oxidizing enzyme caused by 200 r of x-irradiation. To obtain additional information on the ability of MEA to prevent the radiation-induced inhibition of the development of the drug metabolizing enzyme in the liver, 23-day old male rats were given intraperitoneal injections of 200 mgm./kgm. of MEA ten minutes before 400 r of x-irradiation. The animals were sacrificed and a portion of the liver removed for enzyme measurements at frequent intervals for a period of three weeks after x-irradiation. The results of these measurements are presented in Figure 2 where each point on the curves for the irradiated animals is the average of measurements on the livers of at least four animals and each point on the control curve is the average for 8 to 10 animals.

The data in Figure 2 show that 200 mgm./kgm. of MEA given before 400 r of x-ray substantially reduced the degree of radiation-induced inhibition in the development of the phosphorothioate-oxidizing enzyme system in the livers of young male rats. It is apparent, however, that 400 r of x-irradiation has caused a delay in the development of the enzyme system in the MEA-treated animals. Thus it appears unlikely that the radioprotective activity of MEA would be sufficient to prevent the inhibitory effect of higher doses of x-ray.

Influence of 2-mercaptoethylamine (MEA) on the radiation-induced susceptibility of young male rats to O,O-diethyl O-(4-methylthio-m-tolyl) phosphorothioate (DMP). The results of recent studies (9) on the influence of radiation on the development of resistance in young male rats to the acute toxicity of a cholinergic phosphorothioate, O,O-diethyl O-(4-methylthio-m-tolyl) phosphorothioate (DMP) indicated that doses of x-irradiation as low as 100 r markedly inhibit the development of resistance to this agent. The results of experiments presented above illustrate that MEA prevented the radiation-induced inhibition in synthesis of the drug oxidizing enzyme system.

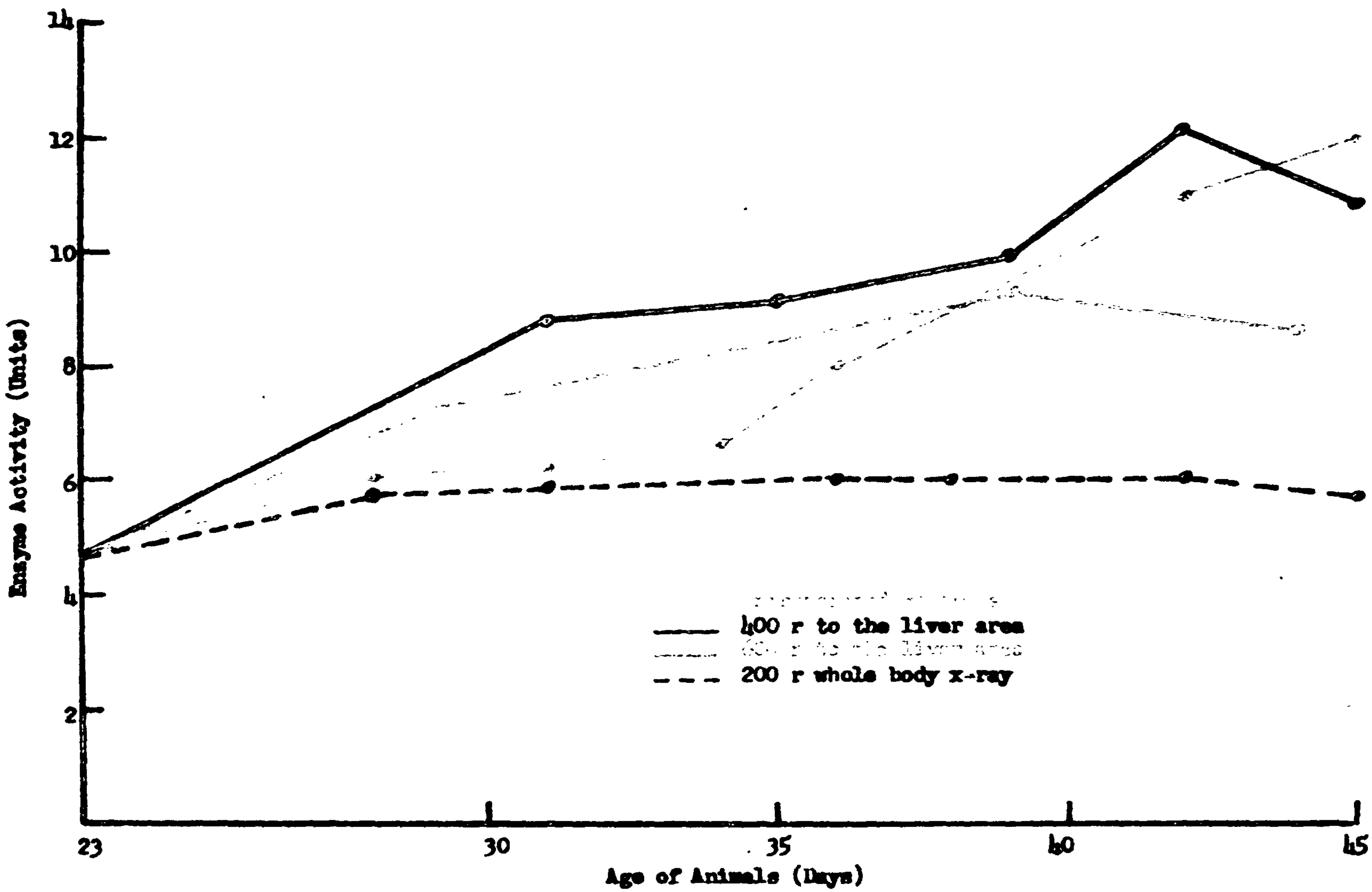

Figure 1. Influence of partial body shielding on the development of the phosphorothioate-oxidising enzyme system in the livers of young male rats.

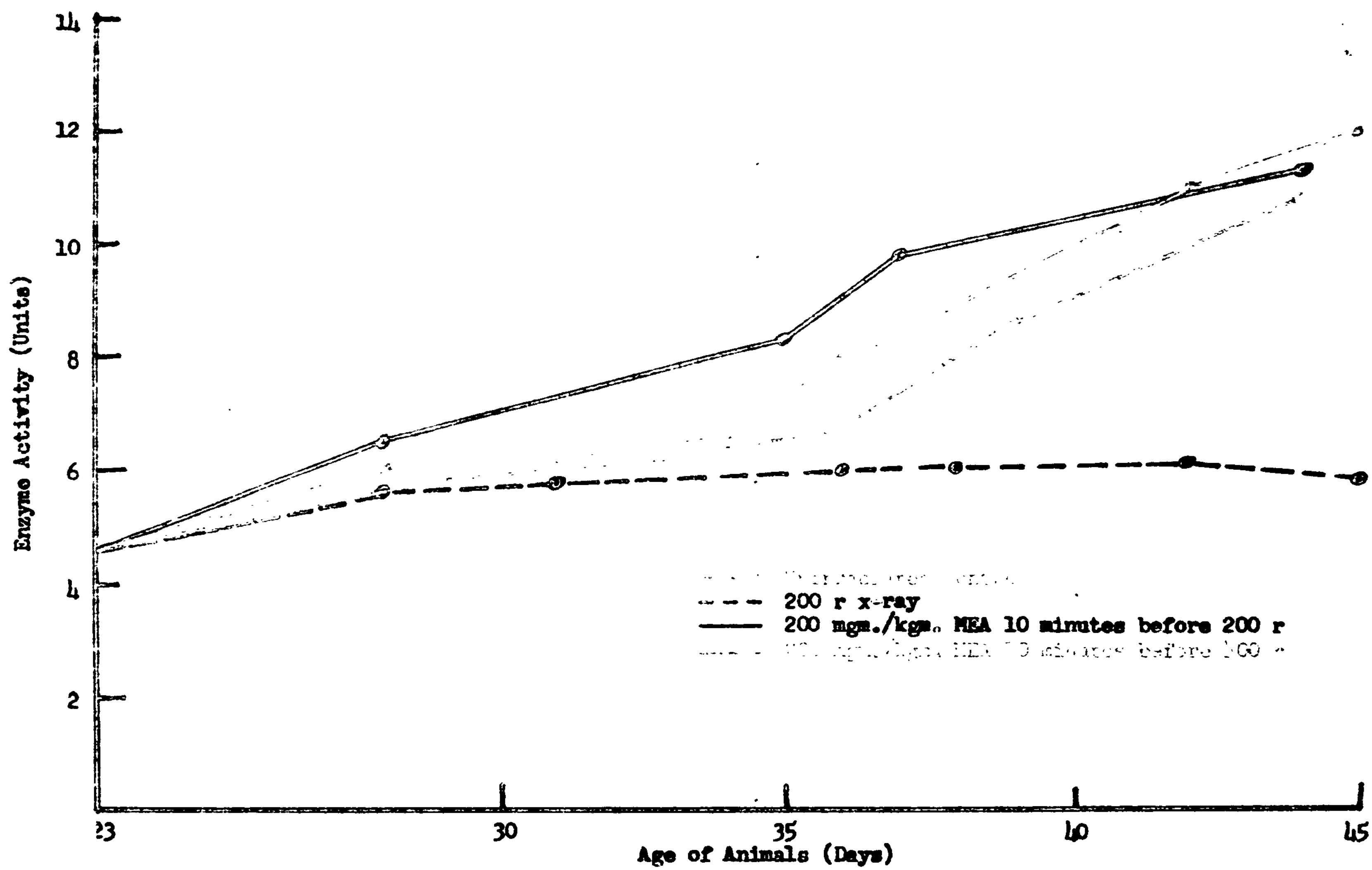

Figure 2. Influence of 2-mercaptoethylamine on the radiation-induced inhibition of the development of the phosphorothioate-oxidizing enzyme system in the livers of young male rats.

Since this enzyme system is involved in the <u>in vivo</u> metabolism of DMP, it was of interest to determine whether MEA would prevent the radiation-induced inhibition in the development of resistance in young male rats to the acute toxicity of DMP. For these experiments groups of 23-day old male rats were given 200 mgm./kgm. of MEA ten minutes before 200 r or 400 r of x-ray. Groups of untreated rats were also given 200 r or 400 r of x-irradiation. Three weeks later the rats were given intraperitoneal injections of 50 mgm./kgm. of DMP. The results of these toxicity tests are presented in Table 1.

TABLE 1

INFLUENCE OF 2-MERCAPTOETHYLAMINE (MEA) ON THE RADIATION-INDUCED SUSCEPTIBILITY OF 44-DAY OLD MALE RATS TO O,O-DIETHYL O-(4-METHYL-THIO-M-TOLYL) PHOSPHOROTHIOATE (DMP)

Dose of X-ray (r)	Dose of MEA (mgm./kgm.)	Dose of DMP (mgm./kgm.)	Survivors/ Treated	% Survival
...	...	50	19/20	95
200	...	..	10/10	100
200	...	50	0/15	0
200	200	50	4/9	44
400	...	..	8/10	80
400	...	50	0/8	0
400	200	50	1/9	11

The data in Table 1 show that most of the unirradiated 45-day old rats tolerated 50 mgm./kgm. of DMP but none of the animals given 200 r or 400 r of x-ray at 23 days of age survived after this dose of DMP. Administration of 200 mgm./kgm. of MEA before 200 r of x-ray permitted four of the nine animals treated to tolerate 50 mgm./kgm. of the phosphorothioate while one of the nine rats given MEA before 400 r survived after this dose of DMP. The results of these experiments indicate that although the development of the oxidizing enzyme system in the livers of young, male, MEA-treated rats is not substantially affected by doses of x-ray up to 400 r, mechanisms responsible for other steps in metabolism of DMP are sensitive to the effects of ionizing radiation and are not completely protected by MEA.

<u>Influence of methyl bis(2-chloroethyl)amine (HN2) on the development of the phosphorothioate-oxidizing enzyme system in the livers of young male</u>

rats. The nitrogen mustards and related alkylating compounds have several actions similar to those produced by x-irradiation. Previous studies in this laboratory have demonstrated the marked similarity between radiation and nitrogen mustards in producing changes in the adenosine triphosphatase activity of the spleen and thymus glands (10), the cholinesterase activity of the intestine (11) and in citric acid synthesis in the spleens and thymus glands of rodents (12). Thus it was of interest to determine the effect of HN2 on the development of the drug metabolizing enzyme system in the livers of young rats. For these experiments groups of 23-day old male rats were given sublethal doses of 0.75 mgm./kgm. or 1 mgm./kgm. of HN2 and the animals were sacrificed for measurements of enzyme activity at frequent intervals for a period of three weeks following these injections. The results of these experiments are presented in Figure 3 where each point on the curves represent the average of measurements on the livers of at least three animals.

The data in Figure 3 show that 0.75 mgm./kgm. of HN2 did not significantly alter the development of the phosphorothioate oxidizing enzyme system in the livers of young rats. Administration of 1 mgm./kgm. of HN2 caused a marked delay in the development of the enzyme system for a period of approximately two and a half weeks after injection. There was a marked increase in the enzyme activity of the livers of these animals during the 18-day to 22-day period after 1 mgm./kgm. of this nitrogen mustard but the activity was still substantially lower than normal at this time.

Discussion

The present investigation consisted of additional experiments in a study which was undertaken to obtain information concerning the influence of ionizing radiations on the development of the enzymes in the livers of young male rats which are responsible for the various steps in the metabolism of certain drugs and toxic compounds. The results of recent studies (4) have shown that the administration of 400 r of x-irradiation to the liver area of partially shielded rats did not inhibit the development of the enzymes responsible for oxidative desulfuration of DPD in this tissue and that 200 mgm./kgm. of MEA given ten minutes before 200 r of x-ray prevented the marked inhibition in the rate of synthesis of this system caused by this dose of radiation. Some of the experiments presented in this report were undertaken to supplement these data. In this connection it was of interest to determine the influence of radiation on the development of the enzymes in the livers of partially shielded rats that received 600 r of x-ray to the liver area and to obtain information concerning the ability of MEA to prevent the inhibitory effect of 400 r of x-irradiation on the synthesis of the enzymes. It was found that the administration of 600 r of x-irradiation to the liver area did not inhibit the initial development of the enzyme responsible for drug metabolism but that the synthesis of the system was substantially inhibited during the third week after x-ray. Administration of 200 mgm./kgm. of MEA before exposure to x-ray substantially reduced the inhibitory effect of 400 r.

In view of the ability of MEA to reduce the inhibitory effect of x-irradiation on the development of the drug metabolizing enzymes of the liver, it was of interest to study the influence of this radioprotective

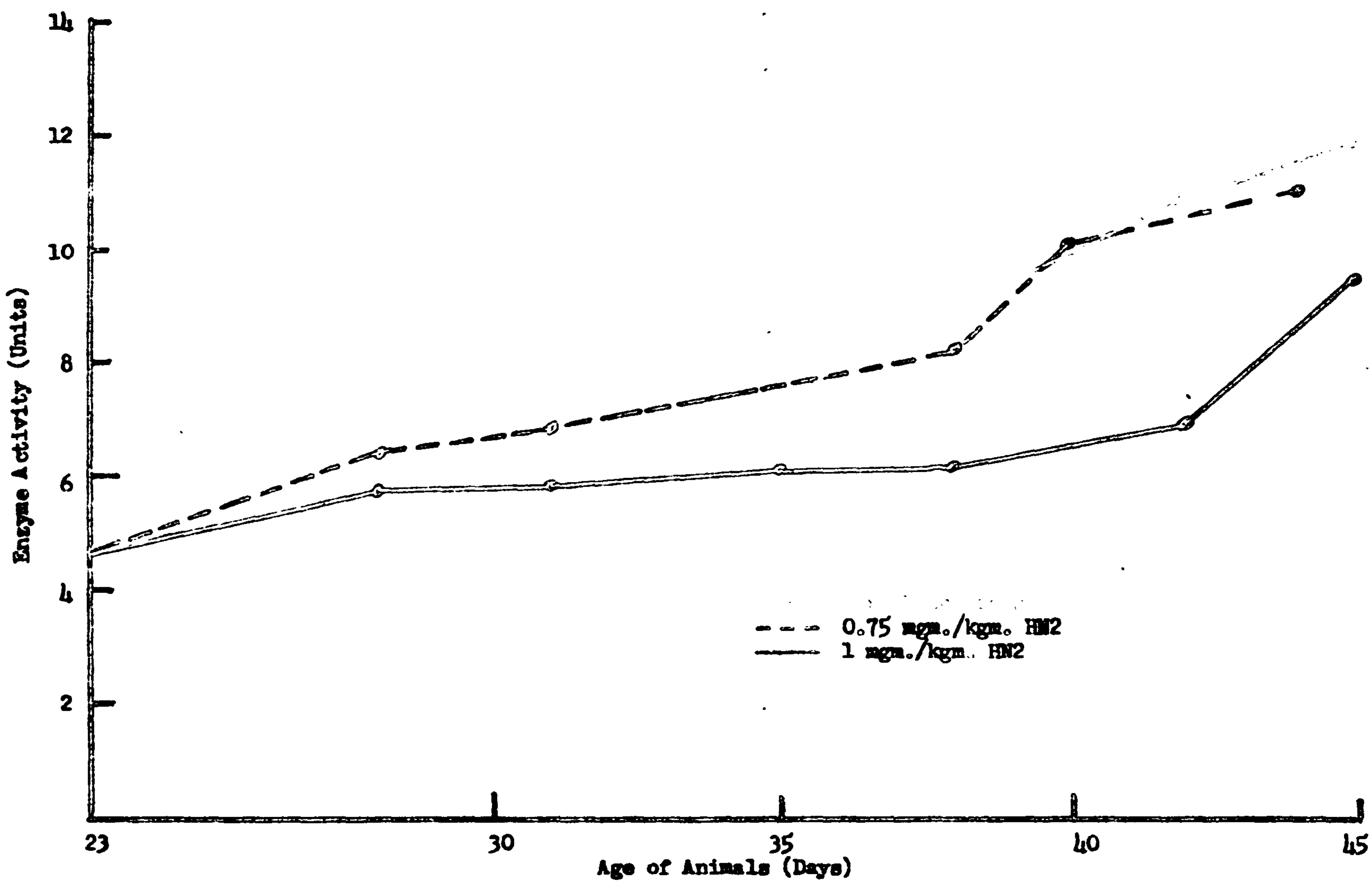

Figure 3. Influence of HN2 on the development of the phosphorothioate-oxidizing enzyme system in the livers of young male rats.

compound on the development of the resistance in young male rats to DMP. Recent studies (13) have shown that DMP and similar compounds undergo an oxidative desulfuration to the corresponding oxygen analogues during an initial phase of metabolism and in this manner exert mammalian toxicity. This reaction is catalyzed by the liver microsome oxidase, the synthesis of which is not affected by the administration of 200 r of x-ray to MEA-treated young rats. Therefore, in order to obtain information concerning the ability of MEA to prevent the inhibition in the development of some of the other mechanisms responsible for the detoxification of DMP, young male rats were treated with 200 mgm./kgm. of MEA before exposure to 200 r or 400 r of x-irradiation. A dose of 50 mgm./kgm. of DMP is usually not lethal to unirradiated 44-day old rats, but this dose of DMP caused mortality in 100% of the unprotected rats given 200 r and in a majority of the animals given MEA prior to 200 r or 400 r of x-irradiation. Thus it is apparent that radiation causes an inhibitory effect on the development of some other mechanism(s) which is necessary for the detoxification of this phosphorothioate. Elucidation of the exact mechanisms affected by radiation remains to be discovered; however, it is anticipated that experiments currently under consideration will provide information which will aid in the localization of the radiation-sensitive site responsible for these findings.

The results of the present experiments have shown that a sublethal dose of nitrogen mustard inhibits the development of the phosphorothioate-oxidizing enzymes in the liver of young male rats. Thus HN2 resembles x-irradiation qualitatively in its ability to depress the synthesis of this enzyme system. This similarity between HN2 and x-irradiation indicates that either of these agents may be employed in future studies undertaken to obtain information on the mechanism responsible for the inhibition of development of the phosphorothioate oxidase in the livers of young male rats.

Summary

1. Additional studies were undertaken to determine the radiosensitivity of the drug metabolizing enzyme system in the livers of young male rats. The results of experiments on the influence of partial body shielding indicated that the administration of 600 r of x-irradiation to the liver area of 23-day old rats appeared to stimulate the synthesis of the phosphorothioate oxidizing enzymes for a period of about ten days after exposure but caused a substantial inhibition in the rate of synthesis during the third week following radiation.

2. The injection of 200 mgm./kgm of MEA ten minutes before 400 r of x-ray provided a substantial reduction in the radiation-induced inhibition of the development of the drug metabolizing enzyme system in the livers of young male rats.

3. Measurements of the effect of MEA on the radiation-induced inhibition of the development of resistance to O,O-diethyl O-(4-methylthio-m-tolyl) phosphorothioate (DMP) were performed. For these measurements 23-day old male rats were given 200 mgm./kgm. of MEA ten minutes before 200 r or 400 r of x-irradiation and were then given intraperitoneal injections of 50 mgm./kgm of DMP at 44 days of age. Results of these acute toxicity

tests showed that 50 mgm./kgm. of DMP, an ordinarily sublethal dose, caused 100% mortality of rats that had been given 200 r or 400 r while 44% and 11% of the animals that had been treated with MEA before 200 r and 400 r of x-ray, respectively, survived after this dose of the phosphorothioate.

4. Results of studies on the influence of nitrogen mustard on the synthesis of the phosphorothioate-oxidizing enzymes indicated that 0.75 mgm./kgm. of HN2 does not affect the normal development of this enzyme system and that 1 mgm./kgm. of this alkylating agent causes substantial inhibition of the rate of development of the drug-metabolizing system.

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THE EFFECTS OF IONIZING RADIATIONS ON THE BIOCHEMISTRY OF MAMMALIAN TISSUES

III. The Influence of Various Chemical Compounds on Radiation-Induced Changes in Enzyme Activities in Certain Rat Tissues

Bernard E. Hietbrink and Marjorie Keshmiri

This report concerns: Additional studies on the influence of drugs and other chemical compounds on the injurious effects of x-irradiation. The present study has been concerned with the quantitative measurement of the effect of sodium pentobarbital (Nembutal) on the radioprotective activity of certain derivatives of dithiocarbamic acid, dimethylammonium dimethyldithiocarbamate (DMDTC) and sodium diethyldithiocarbamate (DEDTC), and of the influence of selachyl acetate, batyl acetate and 2-imino-thiazolidine-4-carboxylic acid on the radiation-induced changes in enzyme activity of the spleen, thymus glands and small intestine of the rat.

Immediate or ultimate application of the results: To obtain information concerning the ability of various chemical agents to reduce the injurious effects of ionizing radiations in the spleen, thymus glands and small intestine of the rat. During the past several months our attention has been directed toward a study of the influence of combinations of chemical agents on the radiation-induced changes in the adenosine triphosphatase activity of the spleen and thymus glands and the cholinesterase activity of the intestine. It has recently been shown that the combination of pentobarbital with 2-aminoethylisothiuronium dibromide (AET) enhances the radioprotective activity of AET and cysteine in rats (1, 2) and monkeys (3). Our recent studies have indicated that in most instances the administration of pentobarbital in combination or with mixtures of the radioprotective agents, 2-mercaptoethylamine (MEA), AET and cysteine failed to enhance the ability of these sulfur-containing agents to reduce the degree of radiation-induced changes in the tissues under investigation (4). The present report contains additional information on the influence of pentobarbital on the radioprotective activity of other sulfur-containing compounds and on the ability of various chemical agents which have not previously been tested for radioprotective activity in rats to reduce the damaging effects of x-ray in the hematopoietic tissues and small intestines. It is anticipated that results obtained from experiments of this nature may be of value in the development of agents and procedures which can be employed to reduce the damaging effects of ionizing radiations.

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Melville et al. (2) have shown that the combination of AET and cysteine is significantly more radioprotective than either agent administered singly and that pentobarbital enhances the protective activity of this combination in rats and monkeys (1, 3). Therefore, in our previous studies (4,5) we were interested in determining the influence of pentobarbital on the radioprotective activity of mixtures of AET and cysteine and of mixtures of other protective compounds in the spleen, thymus glands and small intestine of the rat. The results of this study showed that the administration of pentobarbital before MEA, before or after cysteine or in a mixture with MEA, reduced glutathione or AET and cysteine failed to enhance the protective activity of these agents in the tissues studied. The mixture of 20 mgm./kgm. of pentobarbital and 1,000 mgm./kgm. of cysteine provided marked reductions in the biological effect of 400 r of x-irradiation in the spleen and intestine. Additional studies have recently been undertaken to obtain information concerning the influence of pentobarbital on the radioprotective activity of other sulfur-containing compounds. The results of studies presented in this report illustrate that the administration of pentobarbital in combination with dimethylammonium dimethyldithiocarbamate or sodium diethyldithiocarbamate caused a marked reduction in the ability of these derivatives of dithiocarbamic acid to reduce the injurious effects of x-irradiation in the hematopoietic tissues of rats.

Recent studies (6,7) have shown that various alcoholic substances cause hematopoietic stimulation and have the ability to reduce the lethal effects of ionizing radiations. Thus we have recently undertaken a study (8) to determine the ability of some of these alcohols to moderate the radiation-induced changes in the hematopoietic system and intestine of rats. In initial studies it was found that batyl alcohol causes a marked increase in spleen size in the unirradiated animal (5) and provides a moderate degree of radioprotection in the spleen when given before or immediately after 400 r of x-ray (8). Subsequent studies showed that the administration of 1,000 mgm./kgm. of propylene glycol immediately after 400 r or doses of ethyl alcohol ranging from 200 mgm./kgm. to 500 mgm./kgm. either before or after x-irradiation provided a substantial reduction in the biological effect of x-ray in the spleen. The present investigation consisted of preliminary studies undertaken to ascertain the radioprotective activity of selachyl acetate, an unsaturated alcoholic derivative having a chemical structure similar to that of batyl alcohol, and of batyl acetate. The results of experiments undertaken to determine the influence of 2-imino-thiazolidine-4-carboxylic acid (reaction product of cystine hydrochloride and potassium cyanide) on the radiation-induced changes in the enzyme activities of the spleen, thymus glands and small intestine are also included.

Materials and Methods. Adult, female Sprague-Dawley rats were used for these experiments. The animals were housed in air-conditioned quarters at 68° to 75° F. and were given Rockland Rat Diet and water *ad libitum*. X-irradiation was administered as a single whole body exposure with a G. E. Maximar Therapy unit employing the following radiation factors: 250 KVP, 15 ma., 0.25 mm. Cu and 1 mm. Al added filtration. The target-animal distance was 75 cm. giving a dose rate of 34 r to 36 r per minute as measured in air with a Victoreen ionization chamber. Selachyl acetate and batyl acetate were dissolved in a mixture of 80% propylene glycol and 20% ethyl alcohol. The procedure employed for the preparation of 2-imino-thiazolidine-4-carboxylic acid is presented in detail elsewhere in this progress report (10). All other compounds tested for radioprotective activity were injected as aqueous solutions. In all cases the concentrations were adjusted to permit injections of total volumes not exceeding 1.2% of the body weight.

The adenosine triphosphatase activity of the spleens and thymus glands was measured according to the method of DuBois and Potter (11) using 0.5% homogenates of spleen and 1% homogenates of thymus glands. Assays were performed in duplicate using 0.1 ml. and 0.2 ml. of each aqueous tissue homogenate. Inorganic phosphorus was determined by the method of Fiske and Subbarow (12) and the enzyme activity was expressed as micrograms of phosphorus liberated from adenosine triphosphate by 1 mgm. of tissue during a 15-minute incubation period. The acetylcholinesterase activity of the small intestine was determined by the manometric method of DuBois and Mangun (13). A portion of the small intestine was freed from the mesenteric connective tissue and fat and longitudinally dissected to expel the contents. The tissue was washed with distilled water, blotted with filter paper, minced and homogenized in Ringer-bicarbonate buffer. Measurements were conducted in duplicate using 50 mgm. of tissue per Warburg vessel. The vessels were gassed with 5% CO_2 and 95% N_2 for five minutes. Carbon dioxide evolution was recorded at 5-minute intervals for a period of 30 minutes following a preliminary 10-minute equilibration. Acetylcholinesterase activity was expressed as microliters of CO_2 evolved per 50 mgm. of tissue during a 10-minute incubation period. The degree of radioprotection provided by the chemical compounds in the tissues studied was expressed as per cent reduction of the biologically effective radiation dose. The data presented in this report was calculated using the dose response curves and methods described in a previous report (9).

Results

Influence of sodium pentobarbital on the radioprotective activity of the derivatives of dithiocarbamic acid in the spleen, thymus glands and small intestine of the rat. The influence of the combination of pentobarbital with various sulfur-containing compounds on the injurious effects of radiation in the hematopoietic tissues and intestine of rats has been described in previous reports (4,5). In many instances it was found that pentobarbital reduced the radioprotective activity of these sulfur-containing agents. It was, therefore, of interest to determine whether pentobarbital has a similar influence on the radioprotective activity of the derivatives of dithiocarbamic acid. For these experiments groups each containing four rats were given 25 mgm./kgm. of pentobarbital at various intervals before or after injection of dimethylammonium dimethyldithiocarbamate or sodium diethyldithiocarbamate and then exposed to 400 r of x-irradiation. Three days later the animals were sacrificed for adenosine triphosphatase assays on the spleen and thymus glands and cholinesterase measurements on the intestine. The results of these measurements are presented in Table 1.

The data in Table 1 indicate that the administration of pentobarbital before or after injections of dimethyldithiocarbamate of diethyldithiocarbamate blocks the radioprotective activity of these compounds in the hematopoietic tissues (9). The administration of diethyldithiocarbamate ten minutes before and pentobarbital immediately after 400 r of x-irradiation enhanced the damaging effect of radiation in the intestine.

The influence of various chemical agents on the changes in enzyme activities of the spleens, thymus glands and small intestines of rats three days

TABLE 1

INFLUENCE OF SODIUM PENTOBARBITAL ON THE RADIOPROTECTIVE ACTIVITY OF THE DERIVATIVES OF DITHIOCARBAMIC ACID IN THE SPLEEN, THYMUS GLANDS AND SMALL INTESTINE OF THE RAT

Treatment	Dose in mgm./kgm.	Time of Administration before X-ray	Spleen ATPase[a]		Thymus Glands ATPase[a]		Intestinal Cholinesterase[b]	
			400 r Activity	Effective % Dose Reduction	400 r Activity	Effective % Dose Reduction	400 r Activity	Effective % Dose Reduction
None	...	....	51.4±3.0	..	20.2±1.1	..	69± 9	...
DMDTC Pentobarbital	400 25	10 min. 5 min.	51.1±1.1	0	17.8±2.2	18	62±14	0
Pentobarbital DMDTC	25 400	15 min. 10 min.	48.3±0.5	16	18.0±0.7	16	53± 2	0
DEDTC Pentobarbital	600 25	10 min. 5 min.	53.3±3.1	0	20.7±0.6	0	63± 5	0
Pentobarbital DEDTC	25 600	15 min. 10 min.	52.5±2.6	0	22.9±1.4	0	59± 2	0
DEDTC Pentobarbital	600 25	10 min. ....[c]	52.1±3.3	0	21.5±0.6	0	49± 3	0

[a]Activity expressed as μgm. of P liberated from ATP/mgm. tissue/15 minutes.

[b]Activity expressed as μl. of CO_2 evolved/50 mgm. tissue/10 minutes.

[c]Administered immediately after 400 r of x-irradiation.

after 400 r of x-irradiation. Our previous studies (4,5,8) have indicated that batyl alcohol, propylene glycol and ethyl alcohol provide a moderate degree of protection against the injurious effects of x-irradiation in the spleen. These studies also illustrated that repeated injections of batyl alcohol caused a substantial increase in the size of the spleen. Thus it was of interest to determine the influence of selachyl acetate, an unsaturated compound having a chemical structure similar to that of batyl alcohol, and batyl acetate on the radiation-induced changes in the enzyme activity of the spleens, thymus glands and intestines of rats. For these experiments groups each containing four rats were given doses of 10 mgm./kgm. or 50 mgm./kgm. of selachyl acetate or 30 mgm./kgm. of batyl acetate five minutes before or immediately after 400 r of x-irradiation. Three days later the animals were sacrificed for adenosine triphosphatase assays on the spleens and thymus glands and cholinesterase measurements on the small intestines. The results of these experiments are presented in Table 2.

The data presented in Table 2 illustrate that the administration of 10 mgm./kgm. or 50 mgm./kgm. of selachyl acetate five minutes before or immediately after 400 r provided significant reductions in the biological effect of radiation in the spleens, did not substantially protect the thymus glands and appeared to enhance the effect of x-ray in the intestines. The injection of 30 mgm./kgm. of batyl acetate five minutes before irradiation reduced the degree of radiation-induced change in the adenosine triphosphatase activity of the spleen but had only a slight beneficial effect in the thymus glands and intestine. Batyl acetate did not significantly reduce the damaging effects of 400 r in the hematopoietic tissues and provided a 16% reduction in the biological effect of this dose of radiation when given immediately after x-ray.

The results of experiments presented elsewhere in this progress report (10) show that 2-imino-thiazolidine-4-carboxylic acid, a metabolite formed during the detoxification of cyanide, provides significant protection against the lethal effects of x-irradiation in mice when administered either before or after x-ray. In order to obtain information concerning the ability of this compound to reduce the damaging effects of radiation in the hematopoietic tissues and intestines of rats, 125 mgm./kgm. of the thiazolidine (expressed as equivalents of HCN) was given 25 minutes before or 25 minutes after 400 r of irradiation. The results of these preliminary studies show that 125 mgm./kgm. of 2-imino-thiazolidine-4-carboxylic acid provides significant protection to the spleen when given before x-ray and reduced the degree of radiation-induced change in the spleen and thymus glands when given after 400 r.

Discussion

This investigation consisted of a continuation of experiments undertaken to obtain information concerning the influence of sodium pentobarbital on the radioprotective activity of certain sulfur-containing compounds and to evaluate the degree of protective activity provided the spleens, thymus glands and intestines of rats by various chemical agents. Melville *et al.* (1,2,3) have found that pentobarbital increases the radioprotective activity of the combination of AET and cysteine in rats and monkeys. Our recent studies have

TABLE 2

THE INFLUENCE OF VARIOUS CHEMICAL AGENTS ON THE CHANGES IN ENZYME ACTIVITIES OF THE SPLEEN, THYMUS GLANDS AND SMALL INTESTINE OF RATS THREE DAYS AFTER 400 r OF X-IRRADIATION

Treatment	Dose in mgm./kgm.	Time of Administration with Respect to X-ray	Spleen ATPase[a]		Thymus Glands ATPase[a]		Intestinal Cholinesterase[b]	
			400 r Activity	Effective % Dose Reduction	400 r Activity	Effective % Dose Reduction	400 r Activity	Effective % Dose Reduction
None	...	....	51.4±3.0	..	20.2±1.1	..	69±9	..
Selachyl acetate	10	5 min. before	44.4±2.2	34	18.5±0.5	12	62±4	0
Selachyl acetate	10	Immed. after	45.9±1.3	27	18.9±1.1	10	66±4	0
Selachyl acetate	50	5 min. before	46.2±2.0	26	20.0±1.0	3	62±4	0
Selachyl acetate	50	Immed. after	46.9±3.2	24	20.6±0.2	0	65±1	0
Batyl acetate	30	5 min. before	45.9±2.0	27	19.6±1.3	6	78±6	14
Batyl acetate	30	Immed. after	49.1±2.2	9	22.2±0.8	0	80±11	16
2-imino-thiazolidine-4-carboxylic acid	125[c]	25 min. before	43.4±0.5	37	21.4±0.4	0	62±4	0
2-imino-thiazolidine-4-carboxylic acid	125[c]	25 min. after	44.7±2.2	33	17.9±[illegible].2	18	64±2	0

[a]Activity expressed as μgm. of P liberated from ATP/mgm. tissue/15 minutes.

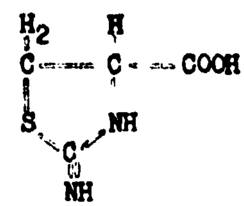
[b]Activity expressed as μl. of CO_2 evolved/50 mgm. tissue/10 minutes.

[c]Expressed as mgm./kgm. equivalents of HCN.

indicated that in most instances the injection of pentobarbital in combination or as a mixture with AET, MEA, cysteine or reduced glutathione does not significantly increase the protective activity of these sulfur-containing agents in the tissues under investigation. The results of the present studies indicate that pentobarbital markedly reduces the radioprotective activity of dimethylammonium dimethyldithiocarbamate and sodium diethyldithiocarbamate in the spleen when it is administered before or after the carbamate derivatives or immediately after x-irradiation.

The results of recent studies (4,5,8) on the influence of batyl alcohol, propylene glycol and ethyl alcohol on the injurious effects of x-irradiation in the spleen, thymus glands and small intestines of rats stimulated our interest in obtaining information on the radioprotective activity of the closely related compounds, selachyl acetate and batyl acetate. The results of preliminary studies indicated that selachyl acetate provided substantial protection to the spleen when given either five minutes before or immediately after 400 r and that batyl acetate causes a significant reduction in the biological effect of radiation when given five minutes before x-ray. Studies are currently in progress to obtain information concerning the influence of these agents on the lethal effects of ionizing radiations.

Studies were undertaken to obtain information concerning the radioprotective activity of 2-imino-thiazolidine-4-carboxylic acid, a metabolite formed in the detoxification of cyanide with the following chemical structure:



This agent is of particular interest because of its structural similarity to AET. The ability of 2-imino-thiazolidine-4-carboxylic acid to protect mice from the lethal effects of x-irradiation is presented elsewhere in this report (10). Administration of 125 mgm./kgm. of thiazolidine 25 minutes before or after x-ray provided substantial reductions in the biological effect of 400 r in the spleen. In view of the preliminary nature of these results, it is apparent that additional studies are required to more accurately evaluate the radioprotective activity of this compound in rats.

Summary

1. A study was conducted to determine the influence of sodium pentobarbital on the radioprotective activity of dimethylammonium dimethyldithiocarbamate (DMDTC) and sodium diethyldithiocarbamate (DEDTC) in the spleens, thymus glands and small intestines of rats. Administration of 25 mgm./kgm. of sodium pentobarbital five minutes before or after DMDTC or DEDTC markedly reduced the radioprotective activity of these derivatives of dithiocarbamic acid.

2. The influence of selachyl acetate and batyl acetate on the changes in the enzyme activities of the spleen, thymus glands and intestine of the rat was investigated. It was found that the injection of 10 mgm./kgm. or 50 mgm./kgm. of selachyl acetate five minutes before or immediately after 400 r of x-ray provided substantial reductions in the biological effect of 400 r in the spleen but did not significantly benefit the thymus glands and small intestine. Administration of 30 mgm./kgm. of batyl acetate five minutes before 400 r of x-ray also provided substantial protection in the spleen but only slight reduction in the biological effect of radiation in the spleen was observed when batyl acetate was given immediately after 400 r.

3. Results of preliminary studies indicated that administration of 125 mgm./kgm. of 2-imino-thiazolidine-4-carboxylic acid (expressed as equivalents of HCN) 25 minutes before or 25 minutes after x-irradiation provided 37% and 33% reductions, respectively, in the biological effects of 400 r in the spleen. This treatment failed to reduce the injurious effects of x-ray in the small intestine.

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THE INFLUENCE OF EXPOSURE TO LOW LEVELS OF GAMMA OR FAST NEUTRON IRRADIATION ON THE LIFE SPAN OF ANIMALS

I. Modification and Dosimetry of the Plutonium-Beryllium Neutron Irradiation Facility

J. Doull, A. Sandberg and D. G. Oldfield

This report concerns: A description of certain changes in the existing low-level fast neutron chronic irradiation facility of this laboratory which were made to increase the flexibility and usefulness of the facility in the chronic irradiation programs now under way, and preliminary calculations of the effect of these changes on the dose distribution within the facility.

Immediate or ultimate application of the results: It is planned to use the modified fast neutron chronic irradiation facility described in this report to investigate the pathogenesis of certain chronic irradiation effects and to correlate some of these effects with radiation-induced changes in the life span of mice.

* * * * * * * *

The 200-curie polonium-beryllium point source used in the original chronic fast neutron irradiation studies in this laboratory (1) had two major disadvantages for the present radiation programs. Because of the relatively short half-life of this source, it was necessary to frequently re-adjust the position of the exposure cages in order to maintain a constant dose rate during the exposure period. To eliminate this difficulty, the polonium-beryllium point source has been replaced by a plutonium-beryllium source. The second disadvantage of the previous facility was that the spherical geometry imposed by the point source limited the number of cages and animals which could be irradiated simultaneously at any given dose rate. In the present facility the point source has been replaced by a linear source having a total length of about 86 cm. This permits the exposure cages to be arranged in a nearly cylindrical array, which greatly facilitates the problems of feeding, watering and handling the individual exposure cages. Since the distance of the exposure cages from the line source is less critical in determining the dose rate within the cage than it was for the previous point source, more cages can be included within each of the desired dose level groups. The range of dose rates available from a linear source is not as great as that obtainable from a comparable point source having the same distance limitations, and thus in order to increase the dose rate flexibility of the present facility, the linear source is constructed of ten individual in-line sources rather than a single source. By removing one or more of these sources and adjusting the configuration of the remaining sources, it is anticipated that the present facility can be given more than adequate flexibility for both the present and future chronic irradiation programs of this laboratory.

Physical description of the fast neutron chronic irradiation facility. The underground room which was used to house the previous polonium-beryllium fast neutron facility has been modified to permit installation of the present plutonium-beryllium fast neutron chronic radiation facility. These modifications consist primarily of (1) a new source containment and positioning device, (2) a different type of caging array, and (3) the addition of a timer-controlled source-drive system for beginning and ending neutron exposure. Front and top views of this room are shown in Figures 1 and 2.

Source. The source for the present facility consists of ten individual 10-curie plutonium-beryllium sources, each contained in a tantalum inner container and a stainless steel outer container, each container being welded shut. These ten sources (1.55" OD x 3.39" H) were placed in a stainless steel tube (1.7" OD x 8 feet, type 304, No. 16 BW) after the bottom end of this tube was Heli-arc welded shut. A 10-inch space was left at the top of the source stack and the rest of the tube was filled with a paraffin plug. The top of the tube was then soldered shut. Since the sources varied slightly in their neutron emission, they were arranged in the following manner within the source container:

Source Number	Neutron Emission in n/sec[a]	Position in Container
M-879	1.83×10^7	1 (bottom)
M-877	1.81×10^7	2
M-881	1.80×10^7	3
M-878	1.78×10^7	4
M-880	1.65×10^7	5
M-872	1.68×10^7	6
M-874	1.72×10^7	7
M-873	1.73×10^7	8
M-876	1.83×10^7	9
M-875	1.84×10^7	10 (top)

[a]Measured by the source manufacturer on January 27, 1961.

The tube containing the sources and paraffin plug is attached to the wire hoisting cable and is freely movable within a second stainless steel tube (2.0" OD x 16 feet, type 304, No. 16 BW) which extends from a point six feet beneath the floor of the exposure room to one foot above the roof of this room. To prevent moisture from entering this outer enclosure, the bottom is welded shut and is further enclosed in a 7-foot length of cast iron well-casing which is embedded in concrete. The top of this stainless steel tube is closed with a plug through which the hoisting cable passes. This plug serves to position the inner source-containing tube and is, therefore, adjustable. The source holding device used in this facility thus provides a triple seal against leakage of the plutonium and further insures that if leakage were to occur, the exposure room itself would not be contaminated.

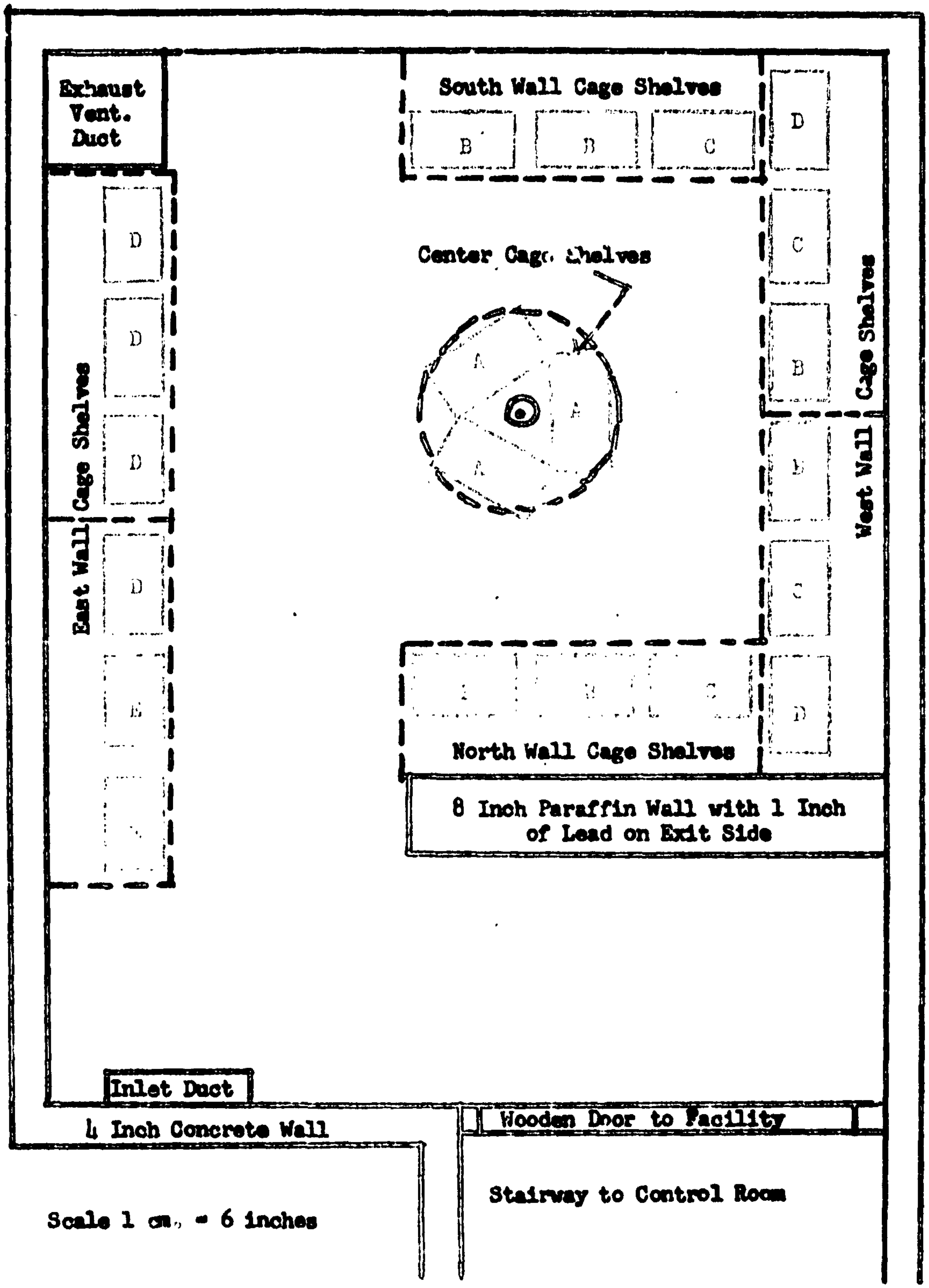

Figure 1. Fast neutron chronic irradiation facility (top plan)

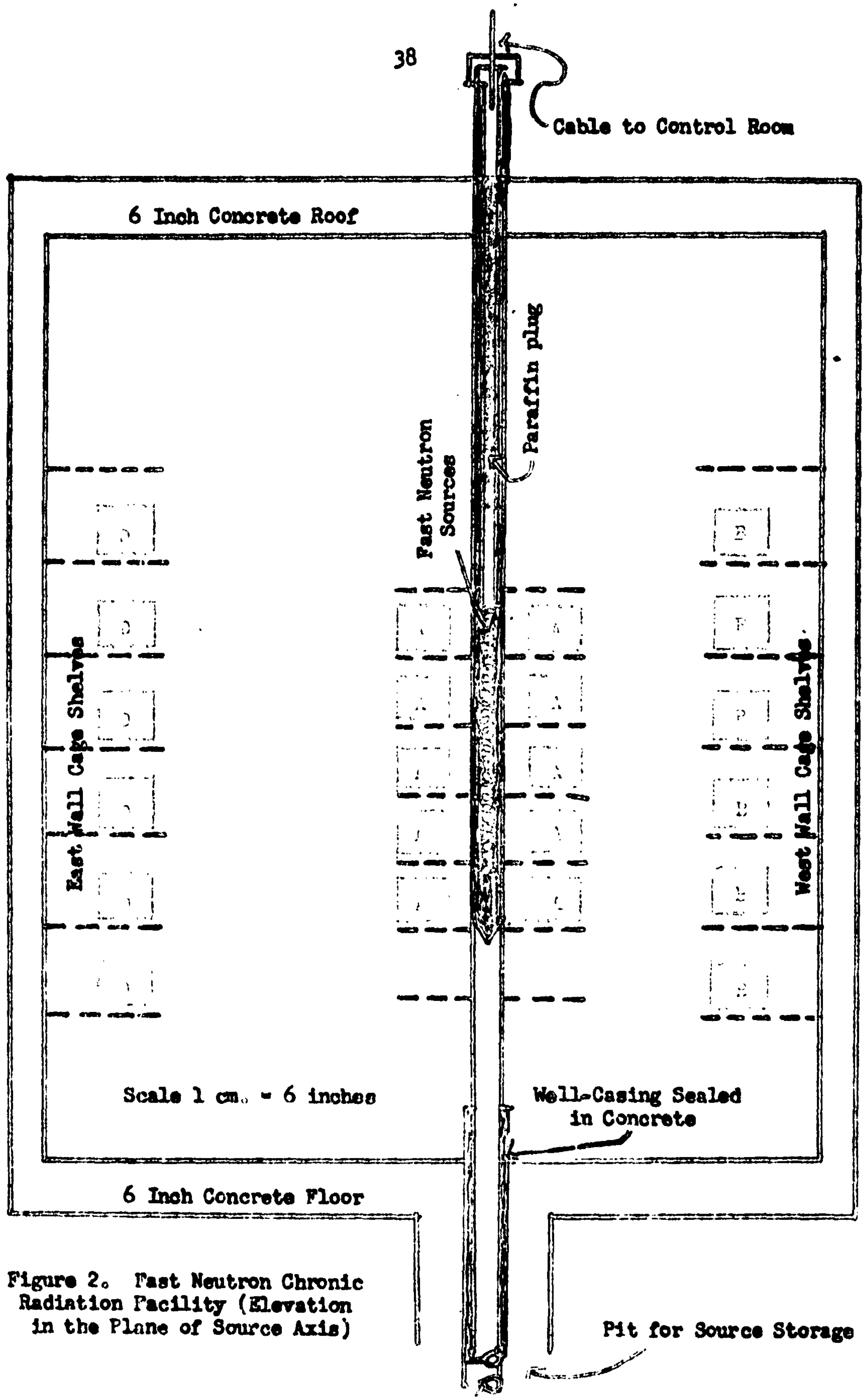

Figure 2. Fast Neutron Chronic Radiation Facility (Elevation in the Plane of Source Axis)

The arrangement of the exposure cages in the present facility is indicated in Figures 1 and 2. Thin-walled, transparent, plastic cages (6.5" x 11" x 5.5") are used to house the animals in this facility. Each of these cages will hold one rat or 8 to 10 mice. The cages are provided with water (four ounce bottle placed on top of each cage) and food and a crushed clay product (Hi-Dri) is used on the floor of the cage to adsorb moisture. One set of these cages (group A in Figures 1 and 2) is cylindrically arranged as close as possible to the source tube and the remaining cages are placed on library type shelves which are attached to the walls of the exposure room. The shelves on the north, south and west walls provide six locations at each level (group B in Figure 1) which are approximately equidistant from the source while the shelves on the east wall provide an additional four positions at each level (group D in Figure 1) which have about the same source-cage distance as the two corner cages on the west wall. Although the shelves on each wall extend from the floor to the ceiling of the exposure room, only the center six levels are being used at the present time to avoid exposing the animals to the extremes in environmental temperature which exist near the floor and ceiling of the exposure room.

In the present facility the source-containing tube is connected to a counterweight by means of a 1/8 inch wire cable. A reversing motor drives the cable between pre-set limits so as to raise or lower the source. Excitation of the motor is controlled by a timer-relay system which permits the duration of each exposure to be pre-set according to any desired daily schedule. An interlock system is provided to prevent the elevation of the source whenever laboratory personnel are in the exposure room, and both visual and audible alarm systems have been provided to indicate whether the source is elevated or within its storage pit. The motor, controlling relays, and timers and safety devices are all located within the control room and are accessible for repair regardless of the position of the source tube. The radiation level in the control and transfer room is continuously monitored by means of a GM tube connected to a count rate meter and recorder. The count rate meter is provided with an alarm system which is activated whenever the radiation level in this area exceeds 20 millirem per hour. Neutron sensitive film badges are worn at all times by the personnel using the facility, and keys to the exposure room are issued only to those individuals directly involved in the chronic radiation programs of this laboratory. A detailed survey by the Health Physics Service of this university indicated that the radiation levels in the control room and in all of the areas surrounding the exposure room were well below the current permissible tolerance levels for both gamma and fast neutron irradiation. These studies also demonstrated that no radiation hazard exists in the exposure room when the source is in the storage pit.

Heating and ventilation of the exposure room is accomplished by means of a forced-air blower system and an exhaust duct connected to an adjacent stack which provides continuous air flow through the room. The temperature in the exposure room is held at 80 ± 3° F. by thermostatic control and is continuously monitored by a strip-chart recorder. The present facility has also been provided with running water to facilitate the daily care of the animals.

<u>Flux and dose calculations for the Pu-Be fast neutron chronic irradiation facility.</u> For biological studies using the fast neutron facility,

several properties of the radiation field produced by the source are of interest. These include: the flux of fast, intermediate, and slow neutrons at any point in the field; the flux of gamma rays in the field and its spectral distribution; the absorbed dose due to each of the above components; the total absorbed dose in the field; and the distribution of LET's producing that dose.

As a basis of comparison with measurements to be reported later, a calculation of the fast neutron flux expected from an ideal line source of neutrons has been made as follows:

A source of overall length L cm. and linear strength S/L neutrons per second per cm. is assumed centered along the z-axis of a cylindrical coordinate system R, ϕ, z. The source is assumed azimuthally (ϕ) symmetric, and the running coordinate of the source is denoted z'. Then the increment of neutron flux dn at the field point R, z due to the source element, S'dz'/L, is

$$dn = \frac{S}{L}\frac{R}{4\pi}\frac{dz'}{\left[(z-z')^2 + R^2\right]^{3/2}} .$$

Integrating between the limits $-\ell$ to $+\ell$, where $\ell = L/2$, we obtain

$$n(R,z) = \frac{S}{4\pi L}\frac{1}{R}\left\{\frac{1}{\sqrt{\dfrac{R^2}{(\ell+z)^2}+1}} \pm \frac{1}{\sqrt{\dfrac{R^2}{(\ell-z)^2}+1}}\right\}$$

The plus sign is taken for ℓ less than z, and the minus sign for ℓ greater than z.

Examination of the function n(R,z) shows that for points near the median plane (z small relative to ℓ), the rate of change of flux with radius, dn/dR, is independent of z for all values of R. Specifically, when R^2 is much smaller than $(\ell - z)^2$, then dn/dR decreases as $1/R^2$; when R^2 is much larger than $(\ell + z)^2$, the dn/dR decreases as $1/R^3$. It can also be shown that, provided z is less than or equal to ℓ, no relative maximum or minimum of n can exist for finite values of R and ℓ. However, when z is greater than ℓ, a relative maximum can exist. Such a maximum occurs when the increase in flux due to increased solid angle exceeds the decrease in flux due to a larger value of R. That is, the flux can increase even if the field point moves radially away from the source, if the field point "sees" more of the source in so moving.

To apply the above calculation to the Pu-Be line source of this facility, the linear strength, S/L, was taken to be the sum of the source strengths of all ten individual sources (1.767×10^8 neutrons/sec.) divided by the total length of the assembled source (86.11 cm.).

The calculations have been carried out over a range of radial distances, R, which include all of the exposure positions available in the present facility, and for those particular values of z which represent the vertical positions of the cages shown in Figure 2. The results of these calculations are shown in Table 1 and plotted in Figure 3. Corrections to the flux calculated here, which future measurements and/or calculations can be expected to provide, concern the following items: (a) the source has a finite diameter, (b) the source strength S/L is not uniform along the length of the source. (The tantalum and stainless steel cans which enclose the individual sources result in a segmentation of the source into active and non-active regions; in addition, the individual sources have a serial order in the assembled source). (c) The source strength S/L may not be independent of the field coordinates R, ϕ, z, at which the source strength is measured as assumed in this calculation. (d) Absorption in the source container tube and source guide tube will decrease the calculated fast neutron flux. (e) Scattering from target material in the radiation field and from the concrete shielding walls may increase the calculated fast neutron flux at certain points.

Regarding item (a), the correction should be important only at field points relatively close to the source axis. For item (b), the correction is easily calculated, and the "tapering" of source strength toward the middle of the source should produce a less rapid decrease in flux as z increases. Regarding item (c), the use of field points having an extreme range in z is not at present contemplated, and no appreciable azimuthal asymmetry is expected. Items (d) and (e) tend to annul each other's effects.

Preliminary measurements carried out using an Eberline PNC-1 paraffin-moderated, cadmium-shielded BF_3 tube suggest that most corrections will be small for all of the present exposure positions. Although these measurements further indicate that the calculated dose rates represent a reasonable approximation of the actual fast neutron dose rates in the present facility, it should be emphasized that the total radiation dosage received by the animals in any exposure position is determined not only by the fast neutron exposure but also by the gamma contaminant from the source and the facility itself. The gamma contaminant of the present facility has not been determined but that of the source is presumed to be somewhat less than the 0.7 gamma per neutron resulting from polonium-beryllium sources.

Since all of the cage positions in the present facility except those closest to the source (Group A) are at least 60 cm. from the source, the variation in flux resulting from the use of a cylindrical cage array is less than ±20% for any group. The variation for the cages on the east wall (Group D in Figure 1) is less than ±10%. These calculations and preliminary measurements suggest that only relatively small corrections should be needed for most of the exposure cage positions in the facility.

Based on these flux calculations, the neutron dose for the various groups of cages in the median plane (vertical center of source) would be approximately as follows:

Group A	3.18 rad per 10-hour day
Group B	0.47 rad per 10-hour day

TABLE 1

CALCULATED NEUTRON FLUX AT VARIOUS POSITIONS IN THE IRRADIATION FACILITY

R (in cm.)	Z (in cm.)									
	0	3.8	12.4	15.2	22.9	34.3	37.5	41.9	43.1	48.0
2.5	130.19[a]	130.17	130.12	130.07	129.85	127.86	124.71	93.40	65.17	....
10	31.75	31.74	31.54	31.43	30.73	26.94	24.14	18.13	16.19	9.03
20	14.78	14.77	14.49	14.34	13.59	11.17	10.11	8.43	7.94	6.02
30	8.92	8.92	8.67	8.53	7.99	6.60	6.09	5.35	5.13	4.28
40	5.97	5.95	5.79	5.69	5.33	4.50	4.22	3.81	3.70	3.23
60	3.16	3.16	3.08	3.03	2.87	2.54	2.43	2.28	2.23	2.05
80	1.94	1.92	1.89	1.86	1.79	1.65	1.58	1.51	1.49	1.41
100	1.29	1.29	1.27	1.26	1.22	1.14	1.09	1.08	1.06	1.02
140	0.68	0.68	0.68	0.67	0.67	0.67	0.64	0.61	0.61	0.60
180	0.42	0.42	0.42	0.42	0.41	0.41	0.39	0.39	0.39	0.39
220	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.27	0.27	0.27

[a]Neutrons x $10^3/cm^2/sec.$

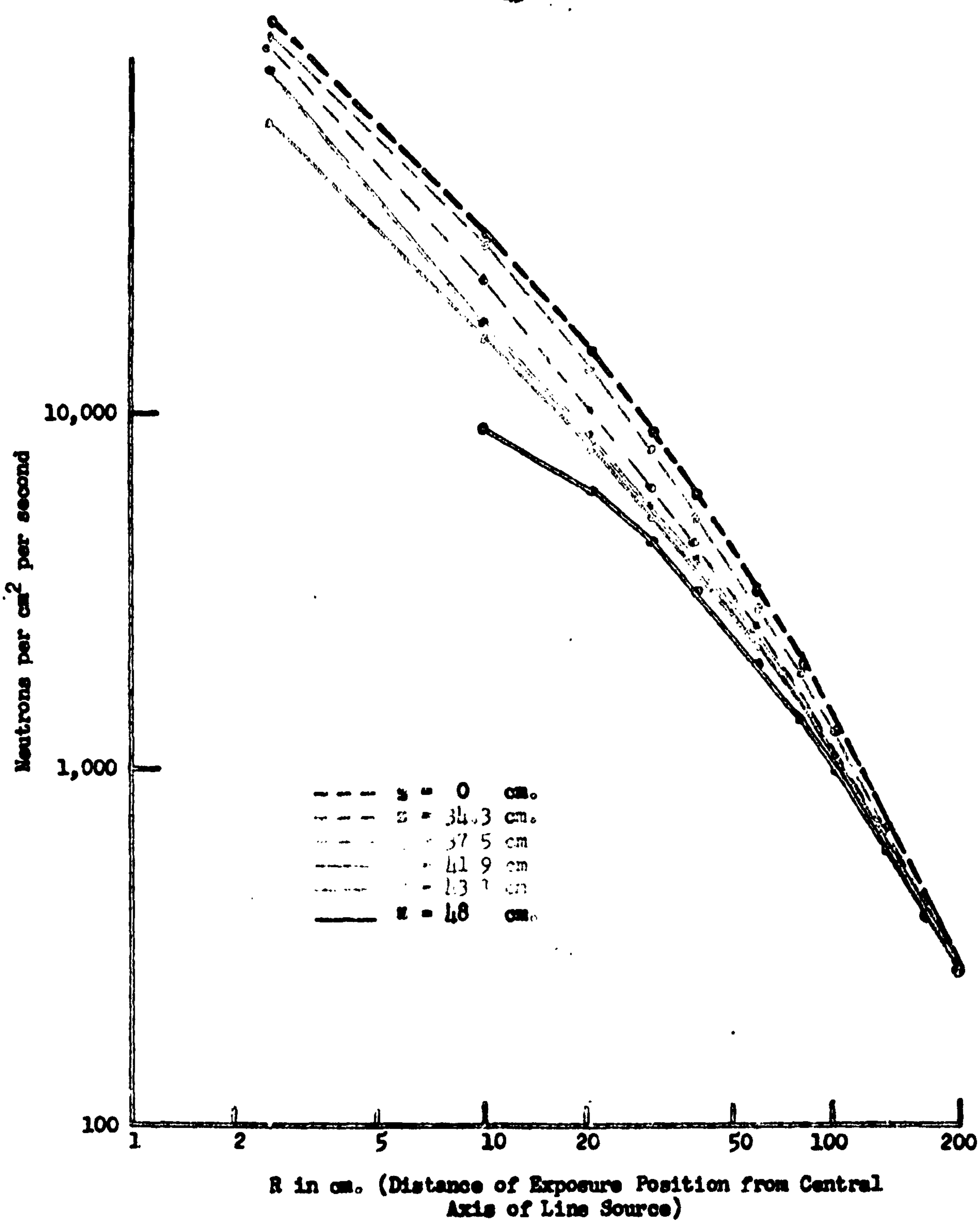

Figure 3. Calculated neutron flux at various exposure positions in facility.

Group C	0.36 rad per 10-hour day
Group D	0.24 rad per 10-hour day
Group E	0.13 rad per 10-hour day

(conversion factor for plutonium-beryllium fast neutrons assumed to be 1.91×10^{-5} rad per hour) (2).

The 25-fold range in dose available with the present cage array could be increased to 100-fold if desired by the use of a somewhat different type of caging around the source and by extending the available cage position space on the east wall by another foot or so. Since these values represent calculated neutron dose rates rather than measured values, it is planned to continue the use of the present cage array at least until measurements of total doses at the various cage positions are completed.

Summary

1. This report contains a description of modifications which have been made in the fast neutron chronic irradiation facility of this laboratory with regard to the type of source, the source configuration, the type of exposure cage and the arrangement of the exposure cages within the facility.

2. These modifications consist primarily in the replacement of the 200-curie polonium-beryllium point source with a 100-curie plutonium-beryllium line source and the use of a cylindrical, rather than a spherical, exposure cage array.

3. Calculation of the neutron flux for the various exposure positions within the modified facility suggest that the modifications have increased the number of animals which can be accommodated simultaneously in the facility and provide a sufficient range in dose rate for both present and future chronic irradiation programs in this laboratory.

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THE INFLUENCE OF EXPOSURE TO LOW LEVELS OF GAMMA AND FAST NEUTRON IRRADIATION ON THE LIFE SPAN OF ANIMALS

II. Effect of Dose Rate on Life Span Shortening of Mice Exposed to Chronic Low Level Fast Neutron Irradiation

A. Sandberg and J. Doull

This report concerns: Survival data for CF_1 female mice exposed to fractionated chronic fast neutron irradiation throughout a duration of life radiation exposure program.

Immediate or ultimate application of the results: To obtain additional information on the injury and recovery processes from chronic radiation exposure. These preliminary studies have permitted a quantitative comparison of the effects of chronic fast neutron irradiation with gamma irradiation for the life span shortening effect produced by irradiation. The effects of chronic irradiation will be determined qualitatively from the histopathological examination of the tissues of mice receiving gamma and fast neutron irradiation. From additional studies now in progress concerning the longevity response to fast neutron irradiation, additional information concerning the effects of variation in dose rate are being obtained which will be of value in determining the environmental hazard associated with fast neutron exposure.

* * * * * * * *

Various model systems have been proposed in recent years for predicting chronic radiation injury and numerous mathematical treatments have been applied to chronic radiation survival data. In order to determine such effects as life span shortening, additional information is needed regarding the changes in survival pattern as a function of dose rate and exposure pattern.

In previous studies in this laboratory concerning the effects of chronic gamma and fast neutron irradiation life span shortening was used as the principal parameter to determine the effects of variation in dose rate, total dose, and exposure pattern (1-4). In the present study a constant exposure pattern has been employed so that the only variables are the dose rate and the total accumulated radiation dose.

In addition to the survival information obtained in this study, animals have been sacrificed at various intervals throughout the experiment so that additional information concerning the pathological findings can be obtained. In the previous experiments in this laboratory histopathological studies were carried out only on animals which had died or were sacrificed in a terminal condition. It is anticipated that the present experiments will provide information concerning the time of onset, rate of progression and incidence of the pathological findings observed in the previous studies (5).

Interim reports have been presented concerning the mortality status of the chronic fast neutron exposure program as well as the complete survival data for the chronic gamma irradiation study (6,7). The present report contains the survival data for the fast neutron irradiated groups throughout the entire duration of life study.

Materials and Methods. Adult, female Carworth Farms CF_1 mice were used for these studies. The animals were between 12 and 16 weeks of age at the beginning of the exposure period on April 17, 1961. The animals were housed in groups of 20 animals per cage in standard stainless steel laboratory cages (9 x 14 x 7 inches) with wire mesh floors. They were provided with food (Rockland Laboratory Mouse Chow) and water *ad libitum*. The irradiated animals were housed continuously in the fast neutron irradiation facility of this laboratory and the control animals were kept in an area which closely approximated the environmental conditions in this facility. Since the animals could be continually housed in the fast neutron facility and irradiated while in the cages in which they lived, no disturbance by additional handling was necessary. The animals were checked daily for mortality and tumor incidence was noted. The temperature in the fast neutron irradiation room and the control room was thermostatically controlled to $80^{\circ} \pm 3^{\circ}$ F.

The chronic fast neutron irradiation exposures were administered by means of a 100-curie plutonium-beryllium source. The average energy of the plutonium-beryllium fast neutrons is 4.5 MEV. The detailed description of this facility, as well as the dosimetry calculations, is given in the preceding section of this report (8). The daily fast neutron exposures were administered over a 9.7 hour time period between 10:00 P.M. and 8:00 A.M. by means of a timing circuit which activated the source-hoisting motor.

Results

Effect of chronic fast neutron irradiation on the life span of CF_1 female mice. Two groups of mice, each of which contained initially 300 animals, were used for these studies. One of these groups was housed in 15 cages which were placed in a semicircle 30 cm. from the line source and the second group of animals was located in cages which were 100 cm. from the fast neutron source.

Preliminary calculations concerning the dose rate for these two groups indicated that the group located 30 cm. from the line source received 1.5 rep/day and that the dose rate for the group located 100 cm. from the source was 0.15 rep/day (7). Further calculations on the dose rate have now been completed and indicate that the high level of neutron irradiated animals received 1.66 rad/day and that the lower level received 0.24 rad/day. These figures are based on the neutron flux in the middle of the cage perpendicular to the cylindrical radius from the center of the in-line source.

The survival data for the neutron irradiated and control groups is shown in Figure 1. The group which received 1.66 rad/day consisted of 300 animals at the initiation of the experiment on April 17, 1961 and the survival data presented here are based on 264 animals, the remaining mice having

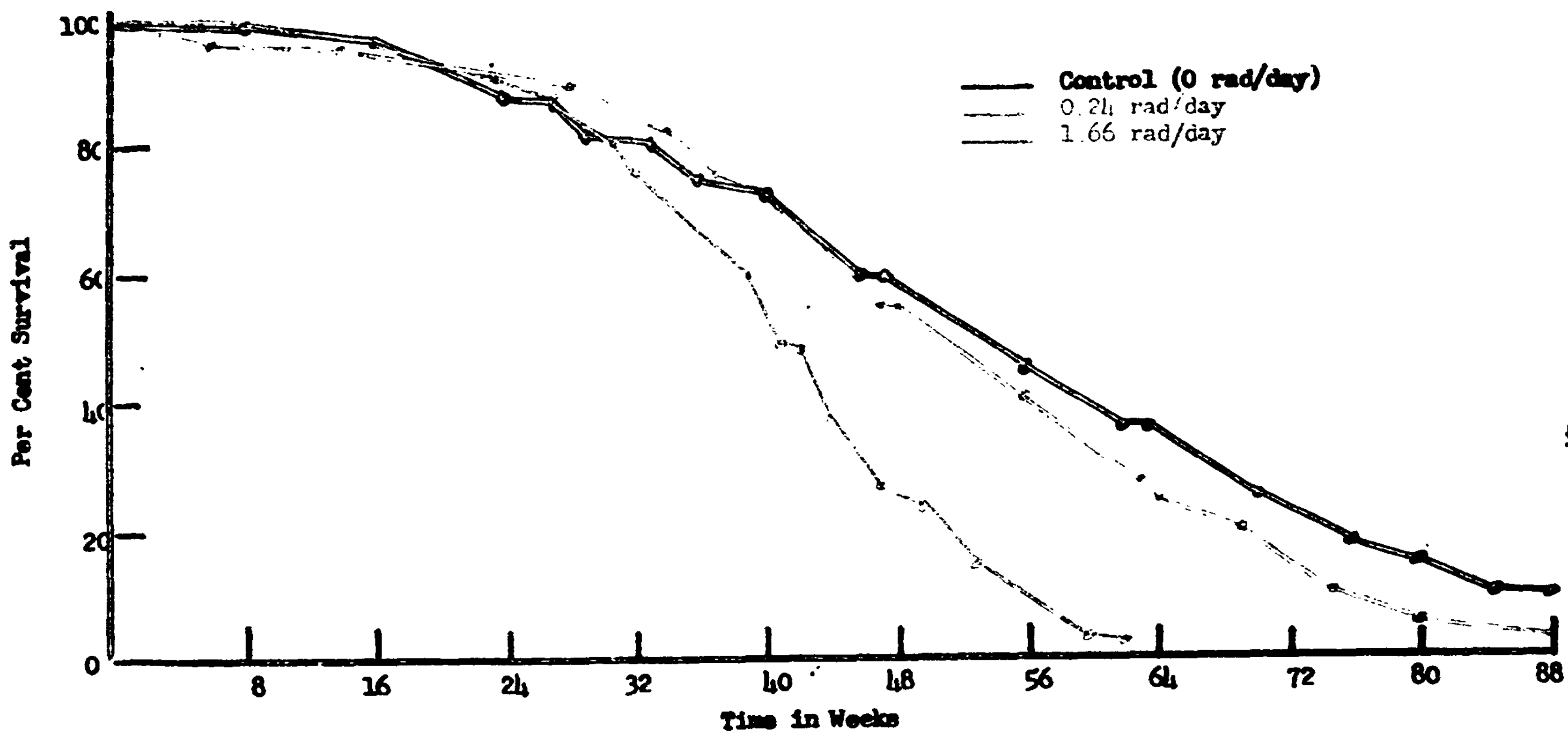

Figure 1. Effect of chronic low level exposure to fast neutron irradiation on the survival of CF_1 female mice.

been sacrificed for histopathological studies during the experimental period. The second group which received 0.24 rad/day also originally consisted of 300 animals and the data for this group are presented for 275 mice, the remainder having been sacrificed. The control group, which was corrected for animals that were removed and sacrificed for the histopathological studies and for the occasional animals which escaped or were inadvertenly killed during cage changing and handling, consisted of 340 animals.

A log probit analysis of the mortality data was used to determine the median survival time (ST_{50}) for each of the irradiation groups and for the control animals. The results of these determinations are shown in Table 1.

TABLE 1

EFFECT OF CHRONIC FAST NEUTRON IRRADIATION ON THE LIFE SPAN OF CF_1 FEMALE MICE

Daily Dose of Fast Neutron Irradiation (rad)	Median Survival Time in Weeks	Life Span Shortening (% of Control)
0 (controls)	53	....
0.24	51	3.7
1.66	41	22.6

The probit transformations are shown in Figure 2. These probit plots approximated straight lines only after an initial 15% mortality. Early deaths before this period straggled out quite irregularly which suggests that there were two different modes of death caused by the chronic fast neutron irradiation. The slopes of the probit analysis curves increase as the radiation dose increases, that of the low level (0.24 rad/day) being greater than that of the control group (0 rad/day) and less than that of the high irradiation level (1.66 rad/day). This would suggest that chronic neutron irradiation not only initiates the natural processes leading to death earlier but once they are initiated they are also accelerated. The histopathological studies will make it possible to determine whether or not the causes of death were essentially the same in the control animals as in those receiving daily neutron exposure.

Although the survival data for the groups of mice exposed to gamma irradiation at doses of 7 rep/day to 88 rep/day have been presented in a previous report (6), the life span shortening as a function of daily dose is presented in this report so that some comparison of the effects of gamma and fast neutron irradiation can be made. This is presented in Figure 3 where it can be seen that the slopes of the lines for gamma and fast neutron irradiation differ. Since the slopes of these curves are not the same as determined

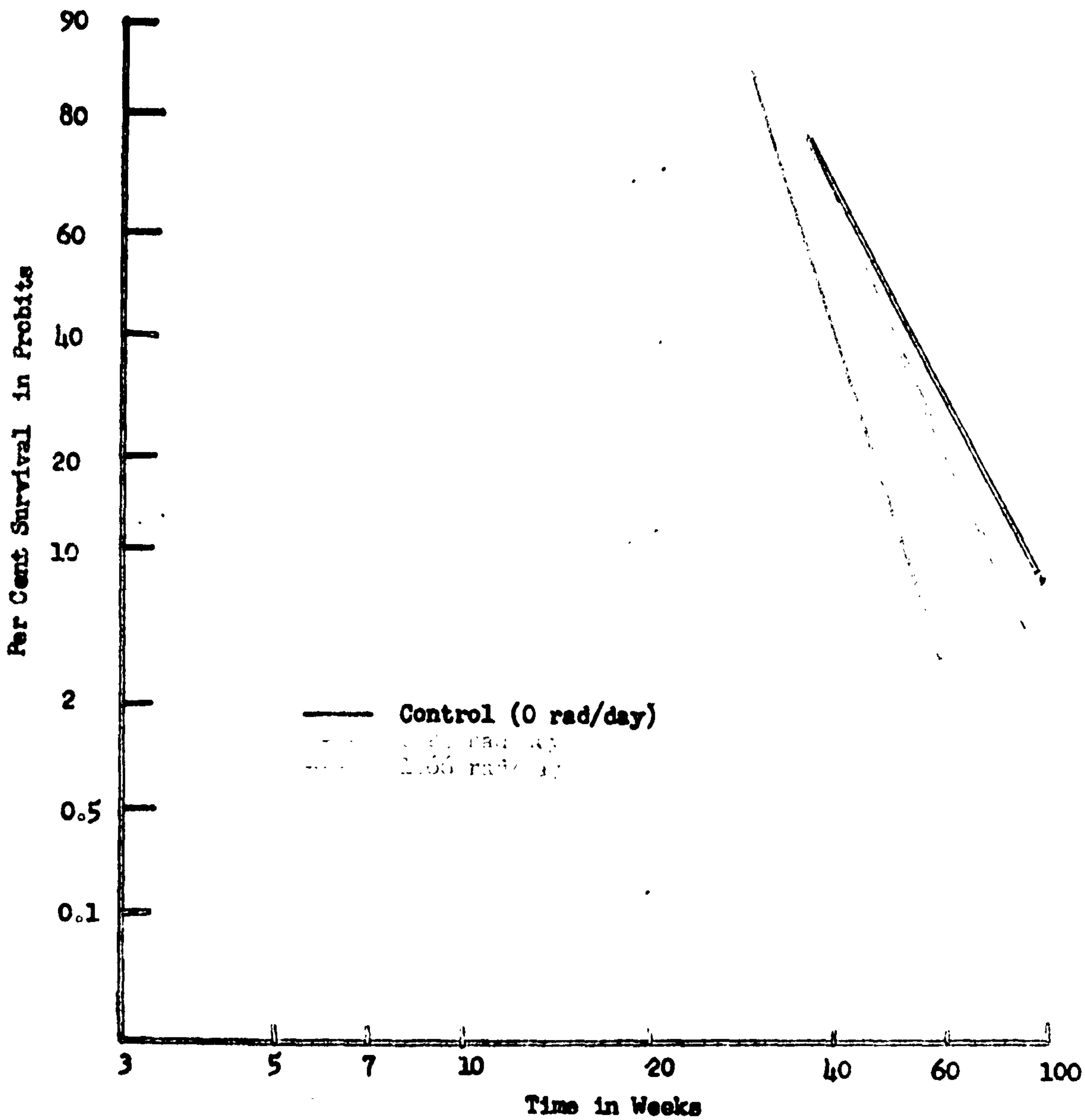

Figure 2. Probit transformation of survival data of CF_1 female mice exposed to chronic fast neutron irradiation.

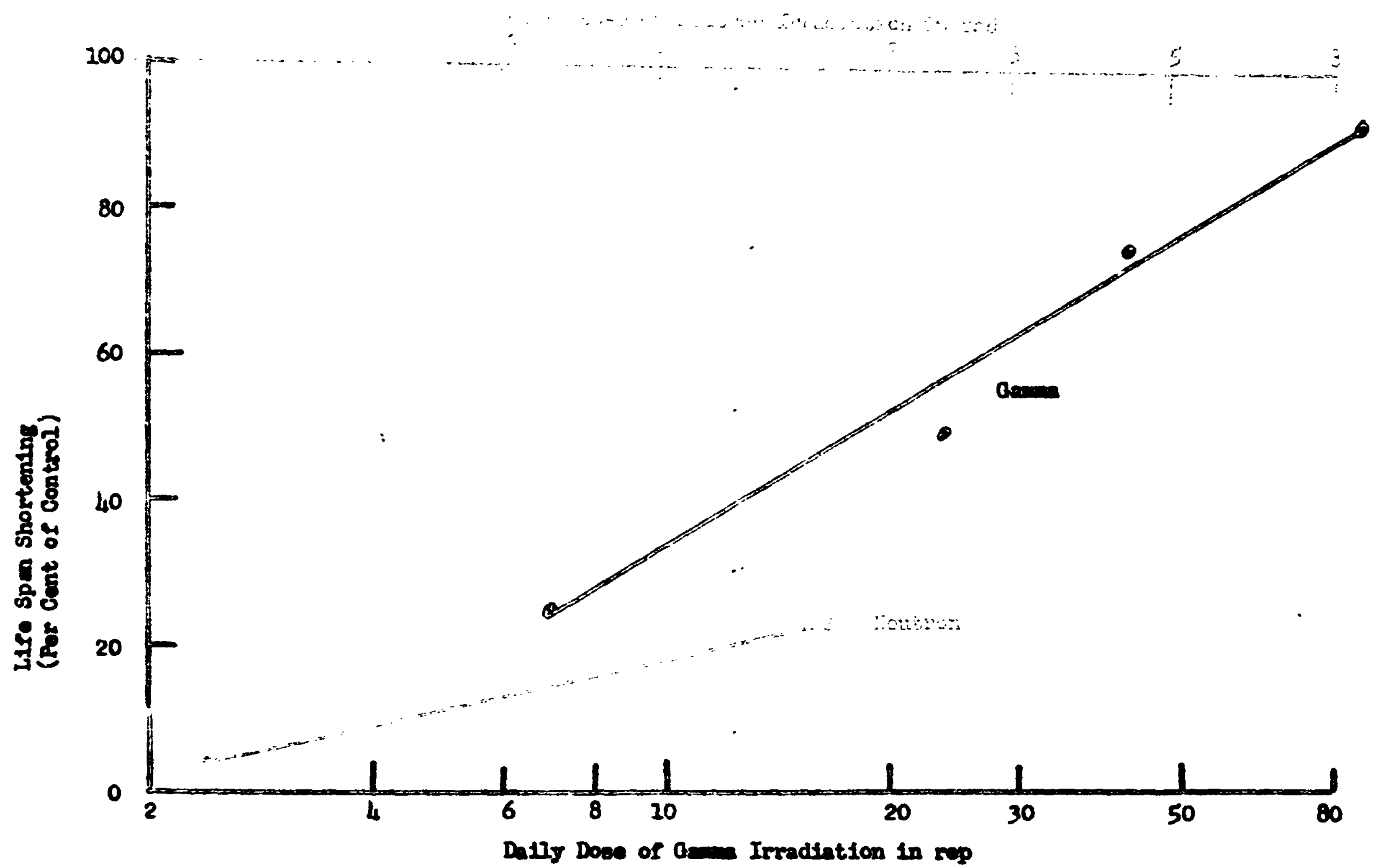

Figure 3. Life span shortening in CF_1 female mice exposed to chronic gamma and fast neutron irradiation.

by the present data, the relative biological efficiency will differ for each per cent life span shortening value, being greater at the lower dose levels and less at the higher dose levels. If the gamma curve is extended to include a life span shortening of 20%, the RBE is 4.8 which agrees well with the value of five determined in previous studies in this laboratory by Noble et al. (1).

Discussion

The present studies in our laboratory concerning the life span shortening effects of chronic fast neutron irradiation have been of a preliminary nature to determine whether the output of the present fast neutron chronic irradiation facility is adequate to produce a wide range of life span shortening. From the data presented in this report and from the calculations concerning dose rates at various positions in the fast neutron facility, it seems likely that this can be attained and the present source is adequate to produce a range of life span shortening of from 0% to about 50% by varying the distance of the animals from the source and by increasing or decreasing the daily exposure time. Since these studies have shown that this facility is suitable for causing a wide range of life span shortening, other groups of animals have been placed in the neutron irradiation room as indicated in the previous section of this report which demonstrates the positioning of the five additional groups of animals added to the study. These groups will make it possible to obtain additional information concerning the effects of dose rate on survival of chronically irradiated animals since additional dosage levels need to be investigated to accurately determine the relationship between the dose rate and life span shortening.

Since the chronic toxicity of irradiation is expected to depend on a balance between the continuing damage produced by the radiation and the ability of the irradiated animals to keep pace with the damage by repair processes, the nature of the injury and the recovery processes in these chronically irradiated animals needs to be investigated to determine their contributions to life span shortening. It is anticipated that the histopathological examination of the tissues of the chronically irradiated mice will give some indication of the nature of this injury.

Since a resemblance between the Gomperts curves for irradiated animals and non-irradiated controls has been observed, it has been postulated that radiation advances the onset of the aging process, but Upton (9) in studying the mortality rate of mice exposed to atomic bomb gamma rays found that the Gompertz curves are not only displaced to the left but are also increased in slope at high dose levels. Strehler and Mildvan (10), in predicting the relationship between the Gomperts slope and radiation exposure, have shown that the Gomertz slope will be increased proportionally to the dose rate for continuous exposure. Although we have not applied the Gompertz analysis to our data, the log probit analysis would suggest that since the slopes increase with increasing daily dose the effects of chronic irradiation do not merely decrease the time of onset of the aging process.

In a recent review concerning experimental studies in the field of ionizing radiation and aging, Upton (9) has compiled data from several investigators concerning the relationship between life span shortening and the log of the dose rate. The data, which we have presented in this report, agrees well with that found by these various investigators concerning both gamma and fast neutron irradiation.

Since at the present time we have survival data on only the two original groups of fast neutron irradiated animals, it is not possible to determine the shape of the curve of life span shortening plotted against the log of the daily dose, but the additional groups of animals which have been added to this study will give some indication of its shape. Although additional dose levels need to be studied regarding gamma irradiation, the results of the present investigations suggest that there is a linear relationship between the life span shortening and the log of the dose rate. Of particular importance is the inclusion of very low dose rates since the results obtained in this region will aid in determining whether a threshold phenomena exists. The present data would suggest that such a threshold phenomena exists for chronic gamma irradiation. Neary et al. (11) have shown that a straight line provides a good fit to their results and one interpretation of this linear relationship is that a threshold of between 1 r and 2 r daily exists below which no shortening of survival time is produced. If a Gaussian curve is fitted to the experimental results, the meaning of the very low doses is less clear.

A comparison of available information on mortality of chronically irradiated mice by Neary et al. (12) suggests that the RBE for this effect is about ten, although we have found in the present study that it varies for different life span shortening values. Vogel et al. (13) have demonstrated that the RBE of gamma irradiation to fission neutrons for acute 30-day lethality is 2.8 and that it is not significantly elevated when the doses are fractionated in 13 daily doses so that several subacute exposures produced effects quite similar to single exposures but differed from those of chronic irradiation.

The findings of these studies and previous studies in this laboratory will be extended when the survival data of the additional groups under study become available and when the histopathological examination has been completed.

Summary

1. Two groups of CF_1 female mice have been exposed to chronic fast neutron irradiation at dose rates of 0.24 rad/day and 1.66 rad/day throughout a duration of life radiation exposure program to obtain information on the life span shortening and histopathological effects of this type of radiation exposure.

2. The median survival time of non-irradiated control mice was 53 weeks, while that of the irradiated groups was 41 weeks for the mice which received 1.66 rad/day and 51 weeks for the mice which received 0.24 rad/day.

3. The group of mice exposed to 0.24 rad/day exhibited a life span shortening of 3.7% while the group which was exposed to 1.66 rad/day exhibited a 22.6% life span shortening.

4. The slopes of the probit analysis curves increase as the radiation dose increases indicating an early initiation of death processes as well as an acceleration following their initiation.

5. The RBE of gamma to fast neutrons as determined by the present data differs for the various per cent life span shortening values, being greater at the low dose levels and decreasing at high dose levels.

6. Additional groups of animals have been placed in the fast neutron facility to obtain additional information concerning life span shortening.

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THE INFLUENCE OF EXPOSURE TO LOW LEVELS OF GAMMA AND FAST NEUTRON IRRADIATION ON THE LIFE SPAN OF ANIMALS

III. Studies on the Toxicity of Rare Earth Compounds and Their Influence on Radiation Lethality

David W. Bruce and Kenneth P. DuBois

This report concerns: Further studies on the acute intravenous toxicity of the rare earth compounds of the lanthanon series when administered alone or in combination with whole body x-irradiation and studies to obtain information concerning their effects on intermediary metabolism.

Immediate or ultimate application of the results: Because of the increase in the industrial utilization of the rare earth compounds, more information is needed on their toxicity. Equally important is the effect of simultaneous exposure to rare earth compounds and ionizing radiations that could result from a nuclear reactor accident. It is anticipated that this program will provide information on the toxicity and biological activity of these compounds and the problems that could arise from simultaneous exposure to radiation and fission products.

* * * * * * * * *

Intravenous toxicity studies on the rare earth nitrates in this laboratory (1,2) have shown that they are highly toxic and that a sex difference exists with respect to the susceptibility of rats to the light lanthanons (cerium, praseodymium, neodymium, and samarium). The ionized salts were found to be 7 to 10 times more toxic to female rats than to males when administered as unneutralized aqueous solutions. Erbium nitrate, a member of the heavy lanthanons, is highly toxic but it does not exhibit a sex difference in toxicity to rats (1).

Sublethal doses of whole body x-irradiation (50 r to 500 r) together with intravenous praseodymium (2 mgm./kgm.) increased the number of observed mortalities 31% to 57% over the mortality resulting from praseodymium alone (1). Studies by Melville and Riess (3) and previous studies in this laboratory have also demonstrated an increase in mortality of rats receiving the rare earth salts intraperitoneally in combination with whole body irradiation. When sublethal doses of the rare earth nitrates were given with 500 r of x-ray, a 27% to 82% increase in mortality was observed (4).

With regard to metabolic studies, intravenous praseodymium (2 mgm./kgm.) was found to have no significant effect on the blood glucose of male rats up to 72 hours after administration; however, in female rats an average decrease of 53% from control values was noted during this time interval. When given with 500 r of total-body x-irradiation, a 29% decrease was observed in 24 hours as compared to a decrease of 19% for praseodymium and 16% for x-ray alone (1). Snyder and Stephens (5) found that intravenous cerium chloride caused a decrease in serum glucose of female rats followed by an increase in plasma free fatty acids suggesting that the first effect of cerium chloride was on carbohydrate metabolism. In addition, we have found that placing female rats on a high carbohydrate diet by the administration of a sucrose-saline solution *ad libitum* prior to intravenous praseodymium caused an apparent reduction in the toxicity of this compound. It also prevented the decrease in the endogenous respiration of liver slices found after the intravenous administration of praseodymium nitrate (6). Our studies, which also indicated that the increased urinary excretion *per se* was not the reason for the decrease in toxicity of this compound, are supported by the studies of Aeberhardt *et al.* (7) and Castellino *et al.* (8) who found that intravenous cerium 144, when given as the ionized salt, is taken up by the liver and excreted by way of the bile with no intestinal reabsorption.

Materials and Methods. Adult, female Sprague-Dawley rats (200 to 250 gm.) were used for these experiments. The animals were housed in air-conditioned quarters and given water and Rockland Rat Diet *ad libitum*. Unneutralized aqueous solutions and colloidal solutions of praseodymium in isotonic saline were given by tail vein. The pH of the unneutralized solution was 5.5 while the colloidal hydroxide prepared by the addition of sodium carbonate to the dissolved nitrate salts was pH 10. Daily administration of 5 mgm./kgm. of testosterone propionate subcutaneously in sesame oil (10 mgm./ml.) was started 30 days prior to the intravenous administration of praseodymium nitrate.

Blood glucose (total reducing value) was determined by the method of Folin and Malmros (9) employing the micromodifications of Park and Johnson (10). Serial samples of whole blood (0.05 ml. in duplicate) were obtained by sectioning the tail under local anesthesia. Tissue slices of rat spleen were prepared using a Stadie-Riggs microtome (11). The slices were suspended in Krebs-Ringer-phosphate buffer (pH 7.4). The endogenous respiration was measured manometrically at 38° C. in an atmosphere of pure oxygen following a 10-minute equilibration period. The QO_2 values were calculated from the dry weight of the tissue slices which were dried to constant weight at 105° C.

X-irradiation was administered as a single, total-body exposure with a G. E. Maximar therapy unit. The radiation factors were as follows: 250 KVP, 15 ma., 0.25 mm. Cu and 1 mm. Al added filtration. The target-animal distance was 75 cm. and the dose rate was 35 r to 37 r/minute as measured in air with a Victoreen ionization chamber.

The nitrate compound used in this study was obtained from Lindsay Chemical Company, West Chicago, Illinois.

Results

The effect of praseodymium nitrate plus 500 r of total-body x-irradiation on the endogenous respiration of spleen slices of female rats. The effect of x-irradiation and praseodymium on the endogenous respiration of spleen slices was measured at 24, 48 and 72 hours after simultaneous administration of 2 mgm./kgm. plus 500 r of x-ray or rare earth and x-ray alone. This combination of the rare earth and radiation had previously been found to cause 100% mortality in female rats (2). The results of this study are shown in Table 1 in which the average and range of values are given for groups containing at least four animals.

TABLE 1

EFFECT OF INTRAVENOUS PRASEODYMIUM AND WHOLE BODY X-IRRADIATION ON THE ENDOGENOUS RESPIRATION OF RAT SPLEEN SLICES

Treatment	QO_2 Values Hours After Treatment 24	48	72
I.V. saline controls	11.2 (10.6-11.8)	...	...
Saline I.V. plus 500 r x-ray	9.6 (9.4-10.1)	8.5 (7.7-9.1)	8.8 (7.9-9.8)
Praseodymium 2 mgm./kgm. I.V.	12.8 (11.9-14.0)	14.5 (11.7-15.2)	14.6 (12.3-16.5)
Praseodymium 2 mgm./kgm. I.V. plus 500 r x-ray	9.2 (8.9-10.9)	8.5 (7.9-9.2)	8.9 (8.3-9.1)

[a]Praseodymium administered as the nitrate salt to groups containing at least four animals.

The oxygen consumption was decreased 12 to 13% at 24, 48 and 72 hours after 500 r of x-ray. Results obtained by Barron (12) agree with those obtained at 48 and 72 hours after 400 r of whole body x-irradiation as do those of Sullivan and DuBois (13) at 24 and 72 hours. However, a 53% inhibition in endogenous respiration noted by Barron at 24 hours and the 45% inhibition by Sullivan and DuBois at 48 hours was not found in these studies with 500 r of x-ray.

Praseodymium given <u>in vivo</u> as the ionized salt was found to increase the endogenous respiration 11% to 13% at the 24, 48 and 72-hour intervals. The combined effects of radiation and praseodymium were essentially the same as those obtained for radiation alone and the stimulatory effect of praseodymium was masked. Colloidal praseodymium (2 mgm./kgm.) was found to have no effect on the endogenous respiration of spleen slices at 24 or 48 hours after administration. Aeberhardt et al. (7) found that in the case of ionic cerium-144 approximately 0.5% of the administered dose was found in the spleen for the first 72 hours while approximately 4.0% of injected colloidal cerium (pH 10) was found in the spleen during the first 48 hours. If this is true for praseodymium, the next member in the series of lanthanides, it would tend to indicate that although more of the compound is present it is not active when administered intravenously as a colloid.

Influence of whole body x-irradiation on rare earth toxicity. Continuing studies (2) on the influence of varying doses of whole body x-irradiation on mortality in female rats receiving 2 mgm./kgm. of intravenous praseodymium metal are shown in Table 2. Groups containing 5 or 10 animals were given rare earth nitrate or x-ray alone or in combination. Praseodymium was given 10 to 15 minutes prior to whole body irradiation in animals receiving both x-ray and rare earth.

TABLE 2

THE INFLUENCE OF VARYING DOSES OF WHOLE BODY X-IRRADIATION ON THE MORTALITY OF FEMALE RATS GIVEN PRASEODYMIUM NITRATE INTRAVENOUSLY

Treatment	Mortality	% Mortality
Praseodymium nitrate 2 mgm./kgm. I.V.	17/45	37.8
Praseodymium nitrate 2 mgm./kgm. I.V. plus 500 r	20/20	100.0
" " " " plus 400 r	21/25	84.0
" " " " plus 300 r	29/40	72.5
" " " " plus 200 r	15/20	75.0
" " " " plus 100 r	15/20	75.0
" " " " plus 50 r	30/40	75.0

[a]Mortality based upon 30-day observation period.

No mortalities occurred among the irradiated controls at the various dose levels while praseodymium alone caused mortalities in 37.8% of the female rats. The combined effects of the two toxic agents resulted in 72.5% to 100.0% mortality, an increase of 34.7% to 63.2% over those receiving only praseodymium. It is interesting to note that 72.5% or 75.0% of the rats receiving 50 r to 300 r with praseodymium succumbed. The mortality among rats receiving 400 r was 84.0% and among those receiving 500 r it was 100.0%.

Most of the animals died during the 48 to 72-hour period post-injection with only an occasional death noted after 96 hours. Further studies are being conducted to see if a correlation can be obtained by varying the dose of praseodymium.

Influence of intravenous praseodymium nitrate on blood glucose of female rats. Because of the apparent decrease in toxicity of intravenous praseodymium when 10% sucrose-saline was administered ad libitum and because of the decrease in blood glucose in female rats after the administration of praseodymium (2), it was of interest to compare the effects of an approximate LD_{50} (2 mgm./kgm.) and LD_{100} (4 mgm./kgm.) dose of praseodymium during the 72-hour critical time period. In this experiment serial blood glucose determinations were made and the control values were obtained 24 hours prior to rare earth injection. In Figure 1 the effects of 2 mgm./kgm. and 4 mgm./kgm. of praseodymium on blood glucose are plotted as per cent of control values. Each animal served as its own control and each point on the curves represents the average plus or minus the standard deviation for groups each containing four to eight animals.

Throughout the observation period the effect of each dose of the metal on blood glucose decreased proportionally with time. The effect of 4 mgm./kgm. of praseodymium is approximately twice as great at any given time period. The variation in the slope of the plotted curves is very likely a result of the recovery of some of the animals receiving the LD_{50} by the time that the 72-hour blood glucose value was measured. Animals receiving 4 mgm./kgm. did not survive the 72-hour test period and most of them died between 48 and 72 hours. Additional studies are being conducted to further evaluate this effect and the effect of combined whole body x-irradiation on this phenomena.

Effect of testosterone on blood glucose of female rats given intravenous praseodymium nitrate. In a previous study (2) testosterone propionate (5 mgm./kgm.) was given daily by the subcutaneous route for a period of 20 days prior to the intravenous injection of praseodymium. Since this experiment provided evidence of a possible reversal in blood glucose caused by the daily administration of testosterone, it was of interest to extend the period of testosterone administration to 30 days before the administration of praseodymium. As shown in Table 3 testosterone markedly modified or prevented the decrease in blood glucose during the 24 to 72-hour period following praseodymium. The only significant decrease (13%) was noted in one animal at 72 hours. None of the animals showed any outward toxic signs and all survived a 30-day observation period.

Discussion

The results of these studies seem to indicate that the decrease in blood glucose caused by intravenously administered praseodymium is proportional within biologic variation to the dose of the metal during the critical period of time after administration. The mechanism of action of praseodymium has not been elucidated but a prolonged decrease in blood sugar of this magnitude is in itself sufficient to produce mortalities. Reversal by testosterone of the effect of praseodymium on blood glucose and subsequent survival of the animals indicates that testosterone is involved in preventing or modifying the toxic actions of praseodymium and the observed decrease in blood glucose. The involvement of testosterone in the toxicity of praseodymium could account for the 7 to 10-fold increased susceptibility of female rats to the light lanthanons as compared with male rats (1,2). In this connection Snyder et al. (14) have

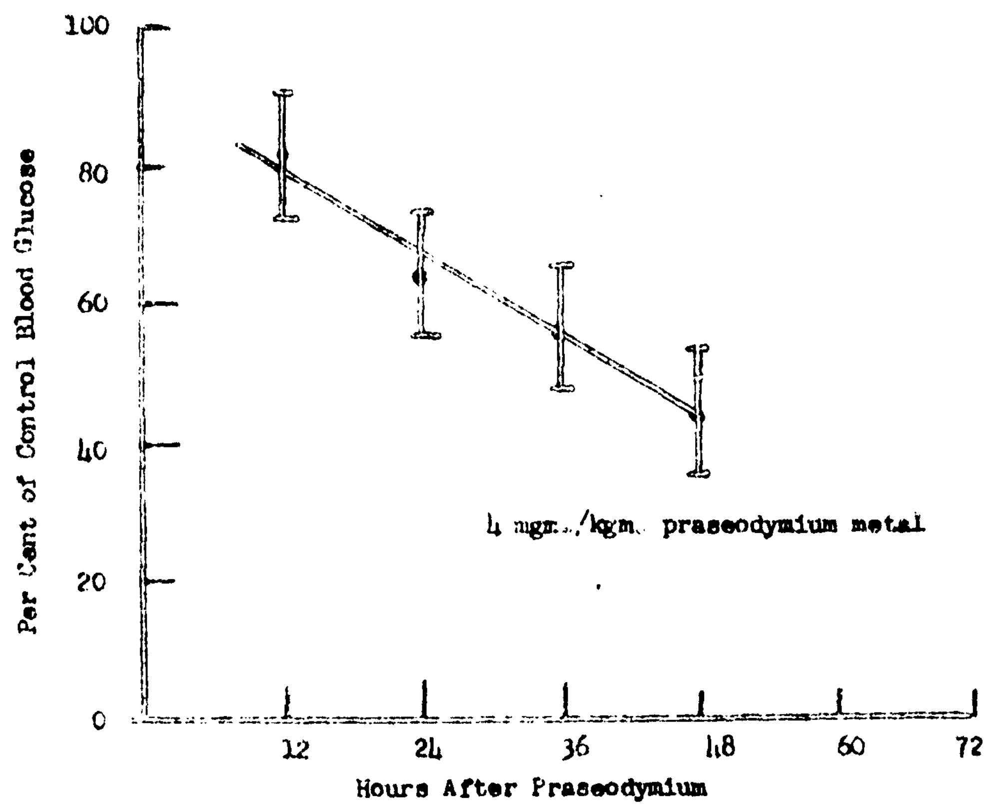

Figure 1. Effect of intravenous praseodymium nitrate on the blood glucose of female rats. Each point represents the average of 4 to 8 animals ± the standard deviation.

TABLE 3

EFFECT OF TESTOSTERONE ON THE BLOOD GLUCOSE OF FEMALE RATS GIVEN INTRAVENOUS PRASEODYMIUM NITRATE[a]

	Blood Glucose (mgm./100 ml.)					
	Control Animals		Praseodymium Metal 2 mgm./kgm. I.V.			
	1	2	3	4	5	6
Control values	105	104	101	118	104	108
24 hours	97	92	92	92	93	94
48 hours	98	92	92	92	89	97
72 hours	108	109	88	108	113	100

[a]Testosterone propionate was administered daily for 30 days prior to the intravenous praseodymium.

found that a given dose of intravenous cerium chloride increased the liver lipid content of castrated male rats but did not affect normal male rats.

The stimulatory effect of intravenously administered praseodymium as the ionized salt on the endogenous respiration of spleen slices cannot be explained on the basis of present knowledge. The studies by Aeberhardt et al. (7) indicate that a very small amount of ionized rare earth is fixed in the spleen and although possibly ten times as much of the colloidal compound is deposited in the spleen, it does not have the stimulatory effect of the ionized salt.

Summary

1. Intravenously administered praseodymium nitrate (2 mgm./kgm.) produced an 11 to 13% increase in the endogenous respiration of spleen slices from female rats during the 24 to 72-hour period post-injection while 500 r of whole body x-irradiation alone and in combination with praseodymium resulted in a 12 to 13% reduction in QO_2 values.

2. A 34% to 63% increase in the toxicity of intravenously administered praseodymium nitrate (2 mgm./kgm.) was observed when given 10 to 15 minutes prior to doses of whole body x-irradiation ranging from 50 r to 500 r.

3. The intravenous administration of 2 mgm./kgm. or 4 mgm./kgm. of praseodymium as the nitrate salt resulted in a proportional decrease with respect to time in the blood glucose of female rats during the 12 to 72-hour period following administration. At any given time during this period, the per cent decrease in blood glucose from control values was approximately twice as great in rats receiving 4 mgm./kgm. of praseodymium.

4. The subcutaneous administration of 5 mgm./kgm. of testosterone propionate for 30 days prior to the intravenous injection of 2 mgm./kgm. of praseodymium prevented or modified the resultant decrease in blood glucose normally seen after the administration of this compound.

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THE INFLUENCE OF EXPOSURE TO LOW LEVELS OF GAMMA AND FAST NEUTRON IRRADIATION ON THE LIFE SPAN OF MICE

IV. Histological Findings in the Blood Vessels of Rats and Mice Exposed to Acute and Chronic X-irradiation While Fed Various Synthetic High Fat Diets

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This report concerns: A comparison of the effects of acute x-ray exposure at dosage levels of 400 r through 600 r with those of chronic x-ray exposure at dose rates of either 20 r or 40 r per day (five days per week for 12 weeks) on the microscopic appearance of the blood vessels of mice and rats and the influence of various dietary regimes on these radiation-induced changes.

Immediate or ultimate application of the results: These studies represent part of a program which was initiated in an effort to obtain information on the nature and causes of acute and chronic radiation injury in the cardiovascular system and the role of this type of injury in the development of other types of radiation injury.

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In previous reports (1-3) we have presented information concerning the food consumption, weight changes, survival time data, serum lipid values and the gross pathological findings in the tissues of mice and rats exposed to acute or chronic x-irradiation while receiving various atherogenic and synthetic high fat diets. The present report contains the histological findings in the tissues of these animals with particular attention being given to the radiation-induced effects in the blood vessels.

Although the injurious effects of radiation exposure on the cardiovascular system have been described by a number of investigators (4-17), others (18-20) have failed to observe such changes in specific parts of the cardiovascular system. Additional information is needed concerning the comparative effects of radiation exposure on the different parts of the cardiovascular system and on the pathogenesis of the injury. There also appears to be little information available on the effects of fractionation of the radiation exposure on this type of radiation injury. In the present studies an attempt has been made to obtain this kind of information by systematically examining the blood vessels of most of the major organs, as well as the aorta and other parts of the cardiovascular system. Furthermore, by comparing the changes in animals exposed to acute or chronic radiation it was hoped that some indication of the pathogenesis of these lesions might be obtained. In an effort to enhance the radiation-induced changes in the cardiovascular system, parallel studies have been carried out in animals fed a control diet and in groups of rats and mice fed diets which have been shown previously to produce atheromatous blood vessel injury. The ability of dietary factors to facilitate the development of atherosclerosis has been demonstrated, but in addition to dietary factors, injury can play an important role in the genesis of atherosclerosis by producing an increased permeability of the arterial wall or thickening of the intima (21). Although radiation exposure might be expected to enhance the blood vessel injury resulting from the dietary factors, it is also possible that under certain experimental conditions less injury might be seen histologically since high-fat diets and particularly substances such as methyl lineolate, ethyl lineolate, cottonseed oil and other fats have been found to increase radioresistance (22-26).

Materials and Methods. Male Sprague Dawley rats between 7 and 12 weeks of age and male Carworth Farms CF_1 mice about 10 weeks of age were used for these studies. The experimental design and the number of animals in each of the dietary and x-ray groups were described in a previous progress report. The methods used for feeding and irradiating each of these groups and the composition and preparation of the diets has been described in previous reports (1,2).

At the end of the 12-week experimental period, all of the surviving animals were anesthetized with ether and bled to death by cardiac puncture. Animals which died during the 12-week experimental period were autopsied and the tissues prepared for subsequent histological examination except in a few cases where autolysis made this impractical. The following tissues were routinely taken for the histological examination: liver, kidney, spleen, thymus, heart, lungs, testis, adrenal gland, lymph nodes, pancreas, sternum and the aorta (as much as was possible to obtain). The tissues were fixed in neutral buffered formalin, imbedded with paraffin (except for the mouse hearts which were imbedded in carbowax) and stained with hematoxylin and eosin. Transverse and frontal frozen sections of the heart were stained with Oil Red O for fat and frozen sections of the aorta (ascending, thoracic and abdominal) of rats and of the kidney and liver of both rats and mice were similarly stained with Oil Red O. During these studies a special effort was made to sample the aorta in as uniform a manner as possible. The samples were taken from the middle of the thoracic aorta and between the bifurcation of the iliac and renal arteries in the abdominal aorta. In each case the sample from nearest the aortic origin was used for the fat stain while the more caudal samples were stained with hematoxylin and eosin. Sections of all of the aortas and most of the hearts were also stained with Gomori's trichrome aldehyde fuchsin for elastic tissue, collagen and smooth muscle. The Picro-Mallory V staining method was used for fibrin and Alizarin Red S was used for calcium in additional sections of the aorta. A few sections were also stained with methylene blue for bacteria or with the Gridley fungus stain. Some sections of the aorta were decalcified with formic acid and stained for fat to detect fatty lesions under calcium deposits.

Results

In an effort to bring out the main histological findings of the present studies, the results have been tabulated according to the location of the blood vessels in terms of the frequency and severity of the lesions.

The results obtained in the two species are considered separately and the present report is confined mainly to the changes which were noted in the cardiovascular system although most of the other tissues were examined microscopically.

Aorta. The effect of the diets and radiation alone and in combination on the aorta of the rats and mice is shown in Tables 1 and 2. In the rats sudanophilia of the aortic intima was seen in the animals fed the Fillios and Wilgram diets and this effect was not appreciably altered by the radiation exposure. The x-irradiation, however, appeared to increase the aortic intimal thickening seen in the rats fed the Thomas diet. There was a slightly increased thrombic tendency in the x-rayed groups. The atherogenic diets resulted in degeneration, atrophy in smooth muscle cells, fibrosis and mild infrequent calcification in the media of the aorta of the rats. There was increased degeneration of the media in the x-rayed animals fed the Wilgram and Fillios diets. Chronic and acute inflammatory cells were observed in the adventitia and the periadventitial fatty tissues of the aorta of the rats fed the Thomas and Wilgram diets. These were observed only in the 40 r per day group of rats fed the Wilgram diet. Chronic x-ray exposure alone caused fragmentation of the outermost elastic lamellae and focal atrophy of smooth muscle cells. In the mice, x-ray exposure alone led to an increased tendency to focal swelling and thickening of the intima. The combination of the Wilgram diet and 40 r per day produced less intimal sudanophilia than the diet alone. Sudanophilia of the aortic intima, intimal thickening and thrombosis of the aorta was observed in the mice fed the Wilgram diet but not in the animals which received any of the other atherogenic or high-fat diets. All of the diets caused degeneration and hyalinization of the aortic media. The mice fed the Thomas diet exhibited necrosis and calcification of the media but this was more marked in the animals fed the Wilgram diet. Lipid deposition in the smooth muscle cells similar to that observed in the aorta of the rats was also seen in the mice. The aorta of the mice fed the Wilgram diet was severely calcified so that no estimation of the degree of sudanophilia in the smooth muscle cells was possible although fat staining of an occasional unselected, decalcified aorta showed that lipid deposition was present. A mononuclear infiltration of the adventitia was seen in mice given 20 r per day concurrently with the Wilgram diet. The fatty degeneration and atrophy of the smooth muscle cells was accompanied by a swelling, breaking of the elastic lamellae and a loss of the regular contours of the aortic media. The chronic x-ray exposure caused degeneration and disappearance of smooth muscle cells and hyalinization of the media in the animals fed the Rockland Mouse Diet and the Nursing Purina Chow. The chronic x-ray exposure decreased the incidence of calcification in the media of the mice fed the Wilgram diet. There was increased hyalinization of the media in the mice fed the Fillios diet and exposed to either 20 r or 40 r of x-irradiation daily.

The effect of the acute x-ray exposure on the microscopic findings in the aorta of mice fed the various atherogenic and high-fat diets is shown in Table 3. The acute x-ray exposures alone did not lead to detectable morphological changes in the intima of the aorta. Furthermore, no changes were detected in the intima of the aorta of the mice fed the various atherogenic diets except for the group fed the Wilgram diet and exposed to the various doses of acute radiation where there was sudanophilia of the aortic intima with focal thickening of the endothelium. In the media of the aorta of the mice fed the Wilgram diet and exposed to either 400 r or 450 r, there was

TABLE 1

FREQUENCY AND SEVERITY OF MAJOR HISTOPATHOLOGICAL FINDINGS IN THE AORTA OF THE RAT

Major Histopathological Findings	Diet	0 r			20 r/Day			40 r/Day		
		Asc.	Thor.	Abd.	Asc.	Thor.	Abd.	Asc.	Thor.	Abd.
Lipid deposition in and on the intima	Thomas	0/7	0/7	0/7	0/8	0/8	0/8	0/6	0/6	0/6
	Wilgram	0/9	0/9	0/9	1/10 a	0/10	0/10	0/10	0/10	0/10
	Fillios	1/5 a	2/6 a,a	1/6 a	0/7	1/7 a	0/7	0/8	0/8	0/8
	Wissler	0/5	0/5	0/5	0/5	0/5	0/5	0/2	0/2	0/2
	Rockland Chow	0/4	0/4	0/4	0/5	0/5	0/5	0/5	0/5	0/5
Swelling and focal proliferation of the endothelial cells	Thomas	0/7	0/7	0/7	3/8 a,b,b	1/8 a	1/8 a	0/6	0/6	1/6 a
	Wilgram	0/9	1/9 a	0/9	0/10	0/10	1/10 a	0/10	0/10	0/10
	Fillios	0/6	0/6	0/6	0/7	0/7	0/7	0/8	0/8	0/8
	Wissler	0/5	0/5	0/5	0/5	0/5	0/5	0/2	0/2	0/2
	Rockland Chow	0/4	0/4	0/4	0/5	0/5	0/5	0/5	0/5	0/5
Thrombosis	Thomas	0/7	0/7	0/7	0/8	1/8 b	1/8 b	0/6	0/6	0/6
	Wilgram	0/9	0/9	0/9	0/10	0/10	0/10	0/10	1/10 a	0/10
	Fillios	0/6	0/6	0/6	0/7	0/7	0/7	1/8 a	0/8	0/8
	Wissler	0/5	0/5	0/5	0/5	0/5	0/2	0/2	0/2	0/2
	Rockland Chow	0/4	0/4	0/4	0/5	0/5	0/5	0/5	0/5	0/5

TABLE 1—Continued

Major Histopathological Findings	Diet	0 r			20 r/Day			40 r/Day		
		Asc.	Thor.	Abd.	Asc.	Thor.	Abd.	Asc.	Thor.	Abd.
Media										
Degeneration and disappearance of the smooth muscle cells	Thomas	0/7	0/7	1/7 b	6/8 a,a,a a,a,a	2/8 a,a	2/8 a,b	1/6 a	1/6 a	2/6 a,a
	Wilgram	3/9 a,a,b	2/9 a,a	2/9 a,a	2/10 a,a	2/10 a,a	1/10 a	0/10	0/10	0/10
	Fillios	0/7	1/7 a	1/7 a	0/7	0/7	0/7	0/8	0/8	0/8
	Wissler	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
	Rockland Chow	0/4	0/4	0/4	0/5	0/5	0/5	0/5	0/5	0/5
Degeneration and increase in the collagen in the media	Thomas	0/7	0/7	0/7	2/8 a,b	1/8 a	2/8 a,a	0/6	0/6	0/6
	Wilgram	2/9 a,b	2/9 a,a	1/9 a	4/10 a,a a,a	6/10 a,a,a b,b,b	4/10 a,a b,b	5/10 a,b,b c,d	5/10 a,a,a b,b	3/10 a,a,b
	Fillios	4/6	1/6 a	1/6 a	4/7 a,b b,b	0/7	1/7 a	5/8 a,b,b b,c	3/8 a,a,a	1/8 a
	Wissler	0/5	0/5	0/5	0/5	0/5	0/5	1/5 c	0/2	0/2
	Rockland Chow	0/4	0/4	0/4	1/5 a	0/5	0/5	1/5 c	0/5	0/5
Calcification of the media	Thomas	0/7	0/7	0/7	0/8	0/8	0/8	0/6	0/6	0/6
	Wilgram	1/9 b	0/9	0/9	0/10	0/10	2/10 a,a	0/10	0/10	0/10
	Fillios	1/6 c	0/6	0/6	0/7	0/7	0/7	1/8 a	0/8	0/8
	Wissler	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/2	0/2
	Rockland Chow	0/4	0/4	0/4	0/5	0/5	0/5	0/5	0/5	0/5

TABLE 1—<u>Continued</u>

Major Histopathological Findings	Diet	0 r			20 r/Day			40 r/Day		
		Asc.	Thor.	Abd.	Asc.	Thor.	Abd.	Asc.	Thor.	Abd.
				Adventitia						
Infiltration of adventitia and paraadventitial fatty tissue with mononuclear cells	Thomas	1/6 a	0/7	0/7	0/8	0/8	0/8	0/6	0/6	0/6
	Wilgram	0/9	0/9	0/9	0/10	0/10	0/10	0/10	0/10	1/10 a
	Fillios	0/6	0/6	0/6	0/7	0/7	0/7	0/8	0/8	0/8
	Wissler	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/2	0/2
	Rockland Chow	0/4	0/4	0/4	0/5	0/5	0/5	0/5	0/5	0/5

a = mild, b = moderate, c = marked, d = very marked.

TABLE 2

FREQUENCY AND SEVERITY OF MAJOR HISTOPATHOLOGICAL FINDINGS IN THE AORTA OF MICE

Major Histopathological Findings	Diet	0 r Thor.	0 r Abd.	20 r/Day Thor.	20 r/Day Abd.	40 r/Day Thor.	40 r/Day Abd.
Intima							
Lipid deposition in and on the intima and smooth muscle cells	Thomas	0/4	0/4	0/3	0/3	0/3	0/3
	Wilgram	3/6 b,b,c	2/5 a,a	2/6 a,b	2/5 a,b	1/6 a	1/5 a
	Fillios	0/4	...	0/4	...	0/2	...
	Wissler	0/7	0/7	0/4	0/4	0/3	0/3
	Nursing Purina Chow	0/8	0/8	0/4	0/6	0/6	0/6
	Purina Chow	0/7	0/7	0/6	0/6	0/8	0/8
Swelling and focal proliferation of the endothelial cells	Thomas	0/4	0/4	0/3	0/3	0/3	0/3
	Wilgram	1/6 b	0/5	0/6	0/5	0/6	1/5 a
	Fillios	0/4	0/4	0/4	0/4	0/4	0/2
	Wissler	0/7	0/7	0/4	0/4	0/3	0/3
	Nursing Purina Chow	0/8	0/8	0/4	1/6 a	1/5 a	0/6
	Purina Chow	0/7	0/7	0/6	0/6	0/8	0/8
Thrombosis	Thomas	0/4	0/4	0/3	0/3	0/3	0/3
	Wilgram	1/5 a	0/5	0/6	0/5	0/6	0/5
	Fillios	0/4	0/4	0/4	0/4	0/4	0/2
	Nursing Purina Chow	0/8	0/8	0/4	0/4	0/6	0/6
	Purina Chow	0/7	0/7	0/6	0/6	0/8	0/8

TABLE 2—Continued

Major Histopathological Findings	Diet	0 r		20 r/Day		40 r/Day	
		Thor.	Abd.	Thor.	Abd.	Thor.	Abd.
		Media					
Degeneration and disappearance of the smooth muscle cells	Thomas	2/4 a,a	1/4 a	0/3	0/3	0/3	0/3
	Wilgram	2/5 a,a	2/5 a,a	1/6 a	1/5 a	0/5	3/5 a,a,a
	Fillios	0/4	0/4	0/4	0/4	0/4	0/2
	Wissler	0/7	0/7	0/4	0/4	0/3	0/3
	Nursing Purina Chow	0/8	0/8	0/4	0/6	0/6	0/6
	Purina Chow	0/7	0/7	0/6	0/6	0/8	0/8
Hyalinization of the media	Thomas	0/4	0/4	0/3	0/3	0/3	0/3
	Wilgram	0/6	1/5 a	0/6	0/5	1/6 a	0/5
	Fillios	1/4 a	0/4	2/4 a,a	0/4	3/4 a,a, a	0/2
	Wissler	0/7	0/7	0/4	0/4	0/3	0/3
	Nursing Purina Chow	0/8	0/8	0/4	0/7	1/6 a	3/6 a,a,a
	Purina Chow	0/7	0/7	0/6	1/6 a	2/8 a,a	2/8 a,a
Calcification of the necrotic media	Thomas	1/4 a	0/4	0/3	0/3	0/3	0/3
	Wilgram	6/6 a,b,b c,c,d	5/5 b,b,c c,d	5/6 a,a,b b,b	4/5 a,b,b d	2/6 a,a	2/5 a,a
	Fillios	0/4	0/4	0/4	0/4	0/4	0/4
	Wissler	0/7	0/7	0/4	0/4	0/3	0/3
	Nursing Purina Chow	0/8	0/8	0/6	0/6	0/6	0/6
	Purina Chow	0/7	0/7	0/6	0/6	0/8	0/8

TABLE 2—Continued

Major Histopathological Findings	Diet	0 r		20 r/Day		40 r/Day	
		Thor.	Abd.	Thor.	Abd.	Thor.	Abd.
Adventitia							
Infiltration of adventitia and paraadventitial fatty tissue with mononuclei cells	Thomas	0/4	0/4	0/3	0/3	0/3	0/3
	Wilgram	0/6	0/5	0/6	2/5 b,b	0/6	0/5
	Fillios	0/4	0/4	0/4	0/4	0/4	0/2
	Wissler	0/7	0/7	0/4	0/4	0/3	0/3
	Nursing Purina Chow	0/8	0/8	0/6	0/6	0/6	0/6
	Purina Chow	0/7	0/7	0/6	0/6	0/8	0/8

a = mild, b = moderate, c = marked, d = very marked.

TABLE 3

FREQUENCY AND SEVERITY OF MAJOR HISTOPATHOLOGICAL FINDINGS IN THE AORTA OF ACUTELY IRRADIATED MICE

Major Histopathological Findings	Diet	400 r		450 r		500 r		550 r		600 r	
		Thor.	Abd.	Thor.	Abd.	Thor.	Abd.	Thor.	Abd.	Thor.	Abd.
Intima											
Lipid deposition in and on the intima	Thomas	...	...	0/2	0/2	...	...	...	...	...	...
	Wilgram	1/4 a	1/4 a	0/2	0/2	...	...	...	...	...	...
	Fillios	...	...	0/2	0/2	...	...	...	...	...	...
	Wissler	0/2	0/2	0/3	0/3	...	...	...	...	...	...
	Nursing Purina Chow	0/5	0/5	0/6	0/6	...	...	...	...	...	...
	Purina Chow	0/6	0/6	0/5	0/5	0/5	0/5	0/6	0/6	0/6	0/6
Swelling and focal proliferation of the endothelial cells	Thomas	...	...	0/2	0/2	...	...	...	...	...	...
	Wilgram	1/4 a	0/4	0/2	0/2	...	...	...	...	...	...
	Fillios	...	...	0/2	0/2	...	...	...	...	...	...
	Wissler	0/2	0/2	0/3	0/3	...	...	...	...	...	...
	Nursing Purina Chow	0/5	0/5	0/6	0/6	...	...	...	...	...	...
	Purina Chow	0/6	0/6	0/5	0/5	0/6	0/6	0/6	0/6	0/6	0/6
Media											
Degeneration and disappearance of the smooth muscle cells	Thomas	...	...	0/2	0/2	...	...	...	...	...	...
	Wilgram	1/4 a	0/4	1/2 a	0/2	...	...	...	...	...	...
	Fillios	...	...			...	...	...	...	...	...
	Wissler	0/2	0/2	1/3 a	0/3	...	...	...	...	...	...
	Nursing Purina Chow	0/5	0/5	0/6	0/6	...	...	...	...	...	...
	Purina Chow	0/6	0/6	0/5	0/5	0/6	0/6	1/6 a	0/6	0/6	0/6

TABLE 3—Continued

Major Histopathological Findings	Diet	400 r[a]		450 r		500 r[b]		550 r[b]		600 r[b]	
		Thor.	Abd.	Thor.	Abd.	Thor.	Abd.	Thor.	Abd.	Thor.	Abd.
Thickening and wrinkling of elastic lamellae	Thomas	...	...	0/2	0/2	...	...	...	...	...	...
	Wilgram	2/4	1/4	0/2	0/2	...	...	...	...	...	...
		a,a	a								
	Fillios	...	...	0/2	0/2	...	...	...	...	...	...
	Wissler	0/2	0/2	0/3	0/3	...	...	...	...	...	...
	Nursing Purina Chow	2/5	2/5	2/6	2/6	...	...	...	...	...	...
		a,a	a,a	a,a	a,a						
	Purina Chow	0/6	1/6	1/5	1/5	0/6	0/6	0/6	0/6	0/6	0/6
			a	a	a						
Calcification of the media	Thomas	...	...	0/2	0/2	...	...	...	...	...	...
	Wilgram	2/4	2/4	0/2	0/2	...	...	...	...	...	...
		b,b	c,c								
	Fillios	...	...	0/2	0/2	...	...	...	...	...	...
	Wissler	0/2	0/2	0/3	0/3	...	...	...	...	...	...
	Nursing Purina Chow	0/5	0/5	0/5	0/6	...	...	...	...	...	...
	Purina Chow	1/6 b	0/6	0/5	0/5	0/6	0/6	0/6	0/6	0/6	0/6

a = mild, b = moderate, c = marked, d = very marked.

[a]In this radiation group neither Thomas nor Fillios diets were used.

[b]In these three radiation groups (500 r, 550 r, and 600 r) only mice fed Purina Chow were used.

degeneration and disappearance of the smooth muscle cells. These effects also were seen in the mice fed the Wissler diet and exposed to 450 r and in the control fed group exposed to 550 r. The mice, which were exposed to 400 r of x-irradiation and subsequently fed the Wilgram diet, exhibited degeneration of the elastic lamellae which was characterized by thickening and wrinkling and loss of the usual parallelism and these effects were also noted in the mice fed the Nursing Purina Chow and the control diet following the administration of 400 r and 450 r of x-ray.

Coronary arteries. The major histological findings in the coronary arteries of the rats fed the various diets while receiving the chronic x-ray exposure are shown in Table 4. In the non-irradiated animals intimal lipid deposition was seen in the sections from the rats fed the Wilgram, Thomas and Fillios diets but not in those from the rats fed the Wissler and control diets. The chronic radiation exposure alone did not lead to detectable morphological changes in the intima of the coronary arteries and there were also no changes in the sections from the rats fed the two high-fat diets (Wissler and Nursing Purina Chow). The combination of chronic radiation exposure and any of the three atherogenic diets, however, resulted in a decrease in intimal sudanophilia although there was focal swelling and thickening of the intima. The rats fed the Fillios diet exhibited lipid deposition in the smooth muscle cells of the media but this was not seen in any of the rats fed the diets in combination with the chronic radiation exposure. In Table 5 the major findings in the coronary arteries of the chronically irradiated mice are summarized. Lipid deposition was seen in the intima of the coronary arteries of the mice fed the Wilgram diet and the high-fat and control diets. X-irradiation increased this effect in the high-fat and control diets but decreased the effect in the mice fed the Wilgram diet. Thrombosis of the coronary arteries was rare in the mice fed the high-fat diet and the combination of this diet with chronic x-ray exposure increased the incidence of thrombosis. Lipid deposition in the smooth muscle of the media was seen only in the mice fed the Wilgram diet. There was calcification of the media in several of the animals fed the Wissler diet, and x-irradiation (40 r/day) resulted in similar effects in the mice fed the Purina Nursing diet, the control diet and the Wilgram diet. The effects of acute x-ray exposure alone and in combination with the atherogenic and high-fat diets is shown in Table 6. The combination of either 400 r or 450 r of x-irradiation with the atherogenic diets produced sudanophilia of the intima and media as well as degeneration, necrosis and calcification of the intima and part of the media. Single doses of x-ray did not cause detectable changes in the coronary arteries of the mice fed the control diet.

Renal arteries. Table 7 shows the major histological findings in the renal arteries of the rats which were fed the various diets while receiving daily x-ray exposures of 20 r or 40 r. In the larger renal arteries of the rats fed the Thomas diet, lipid deposition and calcification were seen in the intima. A few rats fed the Wilgram diet and exposed to chronic radiation exhibited focal intimal thickening and thrombosis. Chronic radiation exposure did not appear to enhance the changes in the rats fed the Thomas diet although some calcification of the renal arteries was observed in these animals. The information in Table 8 shows that there was lipid deposition in the intima and media of the renal arteries of the mice fed the Wilgram diet whereas these effects were not present in the mice fed the control diets. There was less

TABLE 4

FREQUENCY AND SEVERITY OF MAJOR HISTOPATHOLOGICAL FINDINGS IN THE CORONARY ARTERIES OF THE RAT

Major Histopathological Findings	Diet	0 r	20 r/Day	40 r/Day
Intima				
Lipid deposition in and on the intima	Thomas	1/7 a	0/7	0/6
	Wilgram	2/9 a,b	0/10	0/10
	Fillios	1/7 a	0/7	0/9
	Wissler	0/5	0/5	0/2
	Rockland Chow	0/4	0/5	0/5
Swelling and focal proliferation of the endothelium	Thomas	1/7 b	0/7	0/6
	Wilgram	1/9 b	0/10	0/10
	Fillios	1/7 a	0/7	1/9 a
	Wissler	0/5	0/5	0/2
	Rockland Chow	0/4	0/5	0/5
Media				
Lipid deposition in the smooth muscle cells	Thomas	0/7	0/7	0/6
	Wilgram	0/9	0/10	0/10
	Fillios	1/7 a	0/7	0/9
	Wissler	0/5	0/5	0/2
	Rockland Chow	0/4	0/5	0/5

a = Mild; b = moderate.

TABLE 5

FREQUENCY AND SEVERITY OF THE MAJOR HISTOPATHOLOGICAL FINDINGS IN THE CORONARY ARTERIES OF MICE

Major Histopathological Findings	Diet	0 r	20 r/Day	40 r/Day
Intima				
Lipid deposition in and on the intima	Thomas	0/6	0/6	0/5
	Wilgram	4/6 a,a,a,b	2/6 a,a	1/5 a
	Fillios	0/5	0/5	0/4
	Wissler	0/7	1/4 a	0/4
	Nursing Purina Chow	0/8	1/4 a	0/6
	Purina Chow	0/7	1/8 a	1/8 a
Thrombosis	Thomas	0/6	0/6	0/5
	Wilgram	0/6	1/6 a	0/5
	Fillios	0/5	0/4	0/4
	Wissler	1/7 a	0/4	1/3 b
	Nursing Purina Chow	0/8	0/4	1/6 b
	Purina Chow	0/7	0/8	0/8
Media				
Lipid deposition in the smooth muscle cells	Thomas	0/6	0/6	0/5
	Wilgram	1/6 a	0/6	0/5
	Fillios	0/5	0/5	0/4
	Wissler	0/7	0/4	0/4
	Nursing Purina Chow	0/8	0/4	0/6
	Purina Chow	0/7	0/8	0/8
Degeneration and calcification of media	Thomas	0/6	0/6	0/5
	Wilgram	0/6	0/6	1/5 a
	Fillios	0/5	0/5	0/4
	Wissler	1/7 a	1/4 a	1/4 a
	Nursing Purina Chow	0/8	0/4	1/6 c
	Purina Chow	0/7	0/8	1/8 a

a = mild, b = moderate, c = marked, d = very marked.

TABLE 6

FREQUENCY AND SEVERITY OF THE MAJOR HISTOPATHOLOGICAL FINDINGS IN THE CORONARY ARTERIES OF MICE

Major Histopathological Findings	Diet	400 r	450 r	500 r	550 r	600 r
Lipid deposition in the intima and media	Thomas		0/2			
	Wilgram	2/4 a,a	2/3 a,a			
	Fillios		0/3			
	Wissler	0/2	0/3			
	Nursing Purina Chow	0/6	0/6			
	Purina Chow	0/6	0/5	0/6	0/5	0/5
Thrombosis	Thomas		0/2			
	Wilgram	0/4	0/3			
	Fillios		0/3			
	Wissler	0/2	0/3			
	Nursing Purina Chow	1/5 a	0/6			
	Purina Chow	0/6	0/5	0/6	0/5	0/5
Degeneration and calcification of the intima and media	Thomas		1/2 a			
	Wilgram	1/4 a	1/3 a			
	Fillios		0/3			
	Wissler	0/2	1/3 a,a			
	Nursing Purina Chow	1/5 b	0/6			
	Purina Chow	0/6	0/5	0/6	0/5	0/5

a = mild, b = moderate, c = marked, d = very marked.

TABLE 7

FREQUENCY AND SEVERITY OF MAJOR HISTOPATHOLOGICAL FINDINGS IN THE RENAL ARTERIES OF THE RAT

Major Histopathological Findings	Diet	0 r	20 r/Day	40 r/Day
Lipid deposition in and on the intima	Thomas	1/8 a	2/7 a,a	1/6 a
	Wilgram	0/9	0/9	0/9
	Fillios	0/6	0/7	0/8
	Wissler	0/5	0/5	0/2
	Rockland Chow	0/4	0/5	0/5
Swelling and focal proliferation of the endothelial cells	Thomas	0/8	0/7	0/6
	Wilgram	0/9	0/9	1/9 a
	Fillios	0/6	0/7	0/8
	Wissler	0/5	0/5	0/2
	Rockland Chow	0/4	0/5	0/5
Calcification	Thomas	0/8	1/7 a	0/6
Thrombosis	Thomas	0/8	0/7	0/6
	Wilgram	0/9	1/9 a	1/9 a
	Fillios	0/6	0/7	0/8

a = Mild.

TABLE 8

FREQUENCY AND SEVERITY OF THE MAJOR HISTOPATHOLOGICAL FINDINGS IN THE PULMONARY AND RENAL ARTERIES IN MICE

Major Histopathological Findings	Diet	Pulmonary Arteries			Renal Arteries		
		0 r	20 r	40 r	0 r	20 r	40 r
Degeneration and calcification of intima and media	Thomas	0/4	0/3	0/2	0/4	0/3	0/2
	Wilgram	4/6 a,a,a,a	4/7 a,a,a,b	0/5	4/6 a,a,a,a	4/7 a,a,b,b	0/5
	Fillios	0/5	0/4	1/4 a	0/5	0/5	0/4
	Wissler	0/7	0/4	0/4	0/7	0/4	1/4 a
	Nursing Purina Chow	0/8	0/6	0/6	0/8	0/6	0/6
	Purina Chow	0/7	1/8 a	1/8 a	0/7	0/8	0/8
Thrombosis	Thomas	1/4 a	0/3	0/2	0/4	0/3	0/2
	Wilgram	1/6 a	0/7	0/5	0/6	0/7	0/5
	Fillios	0/5	0/5	0/5	0/5	0/5	0/4
	Wissler	0/7	0/4	0/4	0/7	0/4	0/4
	Nursing Purina Chow	0/8	1/6 a	1/6 a	0/8	0/6	0/6
	Purina Chow	0/7	0/8	0/8	0/7	0/8	0/8
Lipid deposition on and in the intima	Thomas	0/4	0/3	1/2 a	0/4	0/3	0/2
	Wilgram	1/6 a	0/7	1/5 a	3/6 a,a,b	3/6 a,a,b	0/5
	Fillios	0/5	0/5	0/4	0/5	0/5	0/4
	Wissler	0/7	0/4	0/4	0/7	0/4	0/4
	Nursing Purina Chow	0/8	0/6	0/6	0/8	0/6	0/6
	Purina Chow	0/7	0/8	0/8	0/7	0/8	0/8
Hyaline arterio-sclerosis	Purina Chow	0/7	1/8 a	1/8 a	0/7	0/8	0/8

a = mild, b = moderate, c = marked, d = very marked.

calcification in the mice fed the Wilgram diet and exposed to the daily x-irradiation but the x-ray exposure increased the degeneration and calcification in the intima and media of the renal arteries of the mice fed the Wissler diet. Single acute x-ray exposure followed by the feeding of the various diets produced the effects summarized in Table 9. Although the x-ray exposure alone did not produce detectable morphological changes in the renal arteries of the control groups, there was lipid deposition in the intima and thickening of the intimal wall in the mice fed the Wilgram diet following an x-ray exposure of 400 r.

Pulmonary arteries. The results of the microscopic observations on the pulmonary arteries of the chronically irradiated rats are shown in Table 10. All of the atherogenic diets produced degeneration and calcification of the intima and media of the pulmonary arteries and these effects appeared to be decreased in general by the chronic x-ray exposures. Conversely the combination of the chronic radiation exposure and the feeding of the atherogenic diets appeared to enhance the changes seen in the mice as shown in Table 8. Although there was lipid deposition, calcification of the intima and media, and thrombosis of the pulmonary arteries in the mice fed the Thomas and Wilgram diets, these effects (thrombosis, lipid deposition) were slightly more marked in the mice exposed to either of the dosage levels of chronic x-ray exposure. The major findings seen in the pulmonary arteries of the acutely irradiated mice are shown in Table 11. There was degeneration and calcification of the intima and media of the mice fed the Thomas and Wilgram diet after x-ray doses of 400 r and also in the mice fed the Purina Nursing Chow after higher radiation doses. Plaque formation was seen in the pulmonary arteries of one animal fed the Wilgram diet following exposure to 400 r of x-ray and there was thickening and hyalinization of the arteries of the mice fed the Thomas diet after acute radiation exposure and also in the mice fed the Nursing Purina Chow diet.

Testicular arteries. It can be seen in Table 10 that the diets alone did not cause morphological changes in the testicular arteries of the rats whereas the chronic x-ray exposure (40 r/day) produced necrosis and calcification of the intima and media of these arteries in a few animals fed the control diet. There was thrombosis in the testicular arteries of the rats fed the Fillios diet and exposed to 40 r/day. In mice acute x-ray exposure in combination with the Wilgram diet resulted in degeneration and calcification of the intima and media of the testicular arteries (Table 12). There was also some calcification in the testicular arteries of the mice fed the control diet after an x-ray exposure of 550 r.

Heart. The major histological findings in the hearts of the rats exposed to chronic x-irradiation and fed the various diets is shown in Table 13. There was a mononuclear inflammation of the myocardium in a few of the rats fed the Fillios diet and the rats on this diet, as well as those on the Thomas diet, had hypercellularity of the valves. Foam cells were seen in the tricuspid valves of the rats fed the Thomas diet and lipid deposition was seen in the interstitial mesenchymal cells of the animals fed the Wilgram diet. When the Wilgram fed rats were also exposed to the chronic x-ray, these effects were not evident and there was also no hypercellularity of the atrioventricular valves. However, the chronic irradiation exposure seemed to produce a patchy necrosis and fibrosis of the myocardium in the rats fed the

TABLE 9

FREQUENCY AND SEVERITY OF THE MAJOR HISTOPATHOLOGICAL FINDINGS IN RENAL ARTERIES OF MICE

Major Histopathological Findings	Diet	400 r	450 r	500 r	550 r	600 r
Lipid deposition in and on the intima	Thomas		0/4			
	Wilgram	4/4 a	0/2			
	Fillios		0/3			
	Wissler	0/3	0/3			
	Nursing Purina Chow	0/5	0/6			
	Purina Chow	0/6	0/5	0/6	0/5	0/5
Thickening of entire wall	Thomas		0/4			
	Wilgram	1/4 a	0/2			
	Fillios		0/3			
	Wissler	0/3	0/3			
	Nursing Purina Chow	0/5	0/6			
	Purina Chow	0/6	0/5	0/6	0/5	0/5
Thrombosis	Thomas		0/4			
	Wilgram	1/4 a	0/2			
	Fillios		0/3			
	Wissler	0/3	0/3			
	Nursing Purina Chow	1/5 a	0/6			
	Purina Chow	0/6	0/5	0/6	0/5	0/5

a = mild.

TABLE 10

FREQUENCY AND SEVERITY OF MAJOR HISTOPATHOLOGICAL FINDINGS IN THE PULMONARY AND TESTICULAR ARTERIES OF THE RAT

Major Histopathological Findings	Diet	Pulmonary Arteries			Testicular Arteries		
		0 r	20 r/Day	40 r/Day	0 r	20 r/Day	40 r/Day
Media--Intima							
Necrosis and calcification of the intima and media	Thomas	1/8 a	1/7 a	0/10	0/9	0/9	0/10
	Wilgram	1/9 a	0/7	0/8	0/6	0/7	0/9
	Fillios	4/6 a,a,a,a	0/7	0/7	0/6	0/7	0/7
	Wissler	0/5	0/5	0/2	0/5	0/5	0/2
	Rockland Chow	0/4	0/5	0/5	0/2	0/5	1/5 a
Thrombosis	Thomas	0/8	0/7	0/10	0/9	0/9	0/10
	Wilgram	0/9	0/9	0/10	0/6	0/9	0/10
	Fillios	0/6	0/7	0/7	0/6	0/7	1/7 b
	Wissler	0/5	0/5	0/2	0/5	0/5	0/2
	Rockland Chow	0/4	0/5	0/5	0/4	0/5	0/5

a = Mild; b = moderate.

TABLE 11

FREQUENCY AND SEVERITY OF THE MAJOR HISTOPATHOLOGICAL FINDINGS IN THE PULMONARY ARTERIES IN MICE

Major Histopathological Findings	Diet	400 r	450 r	500 r	550 r	600 r
Calcification of the intima and media	Thomas		2/3 a,a			
	Wilgram	1/4 b	0/3			
	Fillios		0/3			
	Wissler	0/2	0/3			
	Nursing Purina Chow	0/6	0/6			
	Purina Chow	1/6 b	0/5	0/6	1/6 b	1/6 a
Swelling and focal proliferation of the endothelium	Thomas		0/3			
	Wilgram	1/4 b	0/3			
	Fillios		0/3			
	Wissler	0/2	0/3			
	Nursing Purina Chow	0/6	0/6			
	Purina Chow	0/6	0/5	0/6	0/6	0/6
Thickening and hyalinization of the wall of blood vessels	Thomas		1/3 a			
	Wilgram	0/4	0/3			
	Fillios		0/3			
	Wissler	0/2	0/3			
	Nursing Purina Chow	1/6 a	1/6 a			
	Purina Chow	0/6	0/5	3/6 a	0/6	0/6

a = mild, b = moderate.

TABLE 12

FREQUENCY AND SEVERITY OF THE MAJOR HISTOPATHOLOGICAL FINDINGS IN THE TESTICULAR ARTERIES IN MICE

Major Histopathological Findings	Diet	400 r	450 r	500 r	550 r	600 r
Degeneration and calcification of the intima and media	Thomas		0/4			
	Wilgram	0/3	1/3 b			
	Fillios		0/3			
	Wissler	0/2	0/3			
	Nursing Purina Chow	0/6	0/6			
	Purina Chow	0/6	0/5	0/6	1/6 a	0/6
Thickening of the blood vessel wall	Thomas		0/4			
	Wilgram	0/3	1/3 a			
	Fillios		0/3			
	Wissler	0/2	0/3			
	Nursing Purina Chow	0/6	0/6			
	Purina Chow	0/6	0/5	0/6	0/6	0/6

a = mild, b = moderate.

TABLE 13

FREQUENCY AND SEVERITY OF MAJOR HISTOPATHOLOGICAL FINDINGS IN THE HEART OF THE RAT

Major Histopathological Findings	Diet	0 r	20 r/Day	40 r/Day
Lipid deposition in mesenchymal cells of the myocardium	Thomas	0/8	0/7	0/6
	Wilgram	1/9 a	0/9	0/10
	Fillios	0/6	0/7	0/9
	Wissler	0/5	0/5	0/2
	Rockland Chow	0/4	0/5	0/5
Patchy necrosis and fibrosis of the myocardium	Thomas	0/8	1/7 a	0/6
	Wilgram	0/9	2/9 a,a	1/10 a
	Fillios	0/6	0/7	0/9
	Wissler	0/5	0/5	0/2
	Rockland Chow	0/4	0/5	0/5
Mononuclear inflammation	Thomas	0/8	0/7	0/6
	Wilgram	0/9	0/8	0/10
	Fillios	1/6 b	0/7	0/9
	Wissler	0/5	0/5	0/2
	Rockland Chow	0/4	0/5	1/5 a
Hypercellularity of valve	Thomas	2/8 a,a	0/7	0/6
	Wilgram	0/9	1/9 a	2/10 a,a
	Fillios	1/6 a	0/7	0/9
	Wissler	0/5	0/5	0/2
	Rockland Chow	0/4	0/5	0/5

a = Mild; b = moderate.

Wilgram and Thomas diets. The effects of the chronic x-ray exposure and diet feeding on the hearts of the mice are shown in Table 14. There was also lipid deposition in the heart and mononuclear inflammation of the myocardium in the mice fed the Wilgram diet. All of the atherogenic diets caused calcified infarctoid lesions in the heart which were characterized by necrosis of single muscle fibers or by groups of adjoining bundles. The affected areas in most instances became basophilic and exhibited calcification. Calcified coronary arteries were observed infrequently. There was a mural thrombosis in the right atrium of several of the mice fed the Thomas diet. In the mice which received the chronic x-ray exposure in combination with the control diet there was an increased patchy necrosis with calcification of the myocardium and these animals also had a mononuclear infiltration of the myocardium. Lipid deposition was present in the mesenchymal cells of the myocardium in the mice fed the Wilgram diet in combination with the radiation exposure, but the effects were less marked than in animals exposed to the diet alone. These animals did have increased mural thrombosis, infarctoid lesions in the myocardium and occasional hypercellularity of the tricuspid valves. In Table 15 is shown the findings seen in the hearts of the mice exposed to acute x-irradiation and subsequently fed the various diets. The x-ray exposures alone (control diets) produced mononuclear infiltration of the myocardium and calcified infarctoid lesions. Combination of the acute x-ray exposure with subsequent feeding of the atherogenic diets also resulted in lesions of this type and mural thrombi were present in the sections from the mice fed the Wilgram diet after the various x-ray exposures. Thrombi were also seen in some of the animals fed the control diet, but the incidence was lower. One of the animals fed the control diet after receiving 550 r exhibited hypercellularity of the tricuspid valve.

Discussion

The present report concerns the cardiovascular effects of acute and chronic x-ray exposure and the influence of various atherogenic and high-fat diets on these effects in rats and mice. Those studies demonstrate that dietary factors can alter the histological effects of both acute and chronic radiation injury to blood vessels and conversely that radiation exposure (particularly chronic radiation exposure) can alter the atherogenic effects of certain diets in both rats and mice. These findings support and extend the results of the gross pathological observations and the serum lipid determinations on these animals that were reported previously (3).

The diets used for these studies were selected to represent three atherogenic diets (Thomas and Hartroft, Wilgram and Fillios diets), two high-fat diets (Wissler and Purina Nursing Chow), and a control diet (Rockland Laboratory Chow). Each of the three atherogenic diets have been reported (27-29) to produce arteriosclerotic changes in rats whereas such changes would not be anticipated from any of the other three diets. Since none of the atherogenic diets have been previously used to produce such changes in mice, it was necessary to determine whether any of these diets would be suitable for use in mice and whether the type of lesions produced by these diets in mice resembled those produced in rats. It is apparent from the present study that the Wilgram diet is most suitable for this purpose since the animals fed the Thomas and Fillios diets exhibited severe weight loss

TABLE 14

FREQUENCY AND SEVERITY OF THE MAJOR HISTOPATHOLOGICAL FINDINGS IN THE HEART OF MICE

Major Histopathological Findings	Diet	0 r	20 r/Day	40 r/Day
Lipid deposition in mesenchymal cells of the myocardium	Thomas	0/5	0/3	0/2
	Wilgram	4/6 a,a,a,b	2/6 a,a	1/5 a
	Fillios	0/5	0/5	0/4
	Wissler	0/7	2/4 a	0/4
	Nursing Purina Chow	0/4	0/6	0/6
	Purina Chow	0/7	0/8	0/8
Patchy necrosis and calcification of the myocardium	Thomas	2/5 a,b	1/3 a	2/2 a,b
	Wilgram	1/6 a	1/6 a	2/5 b,c
	Fillios	1/5 a	3/5 a,a,a	2/5 a,a
	Wissler	2/7 a,a	1/5 a	0/4
	Nursing Purina Chow	0/4	0/6	1/6 b
	Purina Chow	0/7	0/8	1/8 a
Infarction of the myocardium	Thomas	0/5	0/3	0/2
	Wilgram	0/6	0/6	1/5 a
	Fillios	0/7	0/4	0/4
	Wissler	0/4	0/6	0/4
	Nursing Purina Chow	0/4	0/6	0/6
	Purina Chow	0/7	0/8	0/8
Mural thrombosis	Thomas	1/5 a	0/3	0/2
	Wilgram	0/6	0/6	1/5 a
	Fillios	0/5	0/5	0/4
	Wissler	0/7	0/4	0/4
	Nursing Purina Chow	0/4	0/6	0/6
	Purina Chow	0/7	0/8	0/8
Focal infiltration with mononuclear cells	Thomas	0/5	0/3	0/2
	Wilgram	2/6 a,a	0/6	0/5
	Fillios	0/5	0/5	0/4
	Wissler	0/4	0/6	1/4 a
	Nursing Purina Chow	0/4	1/6 a	0/6
	Purina Chow	0/7	1/8 a	0/8
Hypercellular valve	Wilgram	0/6	0/6	1/5 a

a = mild, b = moderate, c = marked, d = very marked.

TABLE 15

FREQUENCY AND SEVERITY OF THE MAJOR HISTOPATHOLOGICAL FINDINGS IN THE HEART OF MICE

Major Histopathological Findings	Diet	400 r	450 r	500 r	550 r	600 r
Lipid deposition in mesenchymal cells of the myocardium	Thomas		0/4			
	Wilgram	1/4 a	3/3 a,a,a			
	Fillios		0/3			
	Wissler	0/2	0/3			
	Nursing Purina Chow	0/6	0/6			
	Purina Chow	0/7	0/5	0/6	0/5	0/5
Patchy necrotic calcified lesions in the myocardium	Thomas		2/4 a,a			
	Wilgram	1/4 a	3/3 d			
	Fillios		0/3			
	Wissler	0/2	0/3			
	Nursing Purina Chow	1/6 b	2/6 b,b	1/6 a	1/5 a	1/5 a
	Purina Chow	0/7	1/5 a	1/6 a	1/5 a	1/5 a
Mural thrombosis	Thomas		0/4			
	Wilgram	1/4 a	3/3 a,a,a			
	Fillios		0/3			
	Wissler	0/2	0/3			
	Nursing Purina Chow	1/6 a	1/6 a			
	Purina Chow	0/7	0/5	0/6	0/5	0/5
Mononuclear interstitial infiltration	Thomas		0/4			
	Wilgram	0/4	1/3 a			
	Fillios		0/3			
	Wissler	0/2	1/3 c			
	Nursing Purina Chow	1/3 a	0/6			
	Purina Chow	1/7 a	1/5 a	2/6 a	0/5	1/5 a
Hypercellular valve	Purina Chow	0/7	0/5	0/6	1/6 a	0/5

a = mild, b = moderate, c = marked, d = very marked.

and a marked shortening of their survival time. Furthermore, the lesions seen in the mice fed the Wilgram diet resembled in general those reported by Wilgram (28) in the rat. Although histological comparisons of the lesions seen in the mice fed the Thomas and Fillios diet with those in rats are complicated by the severe effects of these diets in mice, the lesions in the two species have several common features and it is likely that these diets could also be used for studies of the present type with relatively minor modifications.

The major histological findings in the rats fed the atherogenic diets and in the mice fed the Wilgram diet consisted of intimal thickening and sudanophilia, lipid deposition and atrophy of the smooth muscle cells in the media, and fibrosis of the media accompanied by secondary wrinkling and fragmentation of the elastic lamellae and vascular thrombosis. Calcification of the degenerated media of the aorta, coronary, renal and pulmonary arteries was a frequent lesion in mice and it was not observed in rats. Of particular interest was the lipid deposition within the smooth muscle cells of the coronary and renal arteries in the rats fed the Fillios and Wilgram diet. Although Wilgram observed lipid in the media (28), he concluded that it was extracellular. Fillios and his associates have described (29) intracellular fat in older blood vessel lesions but they found that the lipid was confined to the foam cells or other mesenchymal cells. The decrease of smooth muscle cells in the media of the rats fed the atherogenic diets is also of particular interest in that such changes have not been reported previously. All of these changes are consistent with the marked elevation in total serum lipids, cholesterol and phospholipids previously observed in the rats used for these studies and since chronic radiation suppressed these diet-induced effects, it was of interest to determine whether these histological findings were also decreased. The microscopic findings presented in this report demonstrate that most of the lipid-containing lesions observed in the blood vessels, particularly those described as lipomatous, were decreased when the atherogenic diet feeding was combined with chronic x-ray exposure. The incidence of thrombosis, however, appeared to be increased slightly by the radiation exposure. In the mice fed the Wilgram diet the chronic x-ray exposure not only decreased the amount of lipid deposition but also decreased the calcification of the blood vessels seen in the non-irradiated animals fed this diet. Acute radiation exposure alone caused a loss of smooth muscle cells and calcification of the aortic media in mice and the lesions were similar to those described by others (15, 30,31). The combination of acute irradiation exposure and the subsequent feeding of the Wilgram diet produced in mice changes similar to those already described in rats whereas the Thomas diet produced only hyalinization and calcification of the pulmonary arteries.

The accumulation of foam cells in the tricuspid valves of the rats fed the Thomas diet and the finding of similar cells in the spleen and in the lumen of the pulmonary arteries suggests the presence of these cells in the circulation as was recently reported by Renaud and Allard (32). Small scattered foci of necrosis and fibrosis were seen in the heart sections of the rats fed the Thomas and Wilgram diets but true infarction, as described by these authors (27,28), did not occur in the present studies. The patchy areas of necrotic myocardium, which were observed in mice fed the atherogenic diets and the Wissler high-fat diet, were mostly calcified whereas in the rats these areas were predominantly fibrotic. The combination of acute x-ray exposure and feeding of the Thomas diet also resulted in patchy necrosis and calcification of the myocardium. Calcific lesion of the blood vessels and myocardium as seen in the mice in the present study are similar in many respects to the lesions described by Lehr in rats with hyperparathyroidism after sulfathiazol intoxication (33). Domingues has also described nearly identical lesions (characterized by medial necrosis and calcification) in rabbits in which renal injury was produced with uranium nitrate (34). Although the parathyroids were not examined in the present studies and serum levels of calcium or phosphorus were not determined, muscular degeneration and lipid deposition were found in the heavily calcified areas of the aortas when the calcium was removed. It is possible that the focal cardiac necrosis seen in the rats fed the Wilgram diet might also have been due to hypervitaminosis D since calcium deposition has been shown in this condition by several investigators (35-43). The calcification seen in the animals fed the Nursing Purina Chow and possibly also the nephrocalcinosis in these mice may have been due to an increased absorption of the dietary calcium because of the lactose content of the diet (44). Calcification and arteriosclerosis of the aorta resulting from the administration of dehydrotachysterol have been shown by Selye (45) to be greatly increased by the removal of the testis, and it is of interest that testicular atrophy was a common finding in the animals fed the atherogenic diets with and without radiation exposure in the present study.

Summary

1. A comparison of the histological findings in the cardiovascular system of mice and rats exposed to various doses of acute and chronic whole-body x-irradiation has been made and the effects of various atherogenic and high-fat diets on these findings has been investigated.

2. Acute radiation exposure in the dosage range of 400 r through 600 r of x-irradiation caused a loss of smooth muscle cells and calcification of the aortic media, calcification and hyalinization of the pulmonary arteries and a mononuclear infiltration with necrosis and calcification of the myocardium. Hypercellularity of the valves of the heart was also observed.

3. Combination of acute radiation exposure and the feeding of various atherogenic and high-fat diets did not result in a decrease in the sudanophilia or calcification of the blood vessels or any of the other diet-induced effects seen in the cardiovascular system of mice.

4. Chronic radiation exposure at the rate of 20 r or 40 r/day for a period of 12 weeks produced necrosis and fibrosis of the aorta in rats and a mild hyalinization of collagen fibers in mice. Calcification of the coronary and testicular arteries was observed in both species. In the mice intimal lipid deposition was observed in the coronary arteries and there was also a focal mononuclear infiltration in the myocardium.

5. Combined exposure to chronic x-irradiation and the feeding of the various atherogenic diets resulted in a decrease in the incidence and in the severity of the diet-induced blood vessel lesions, particularly the lipomatous changes. This was particularly true in the animals fed the Wilgram diet.

The Wilgram diet-induced calcification of the aorta and other blood vessels in mice was also decreased by the chronic x-ray exposure. However, the incidence of thrombosis in the aorta and other arteries appeared to be increased in the animals exposed to chronic x-irradiation.

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PHARMACOLOGICAL AND TOXICOLOGICAL COMPOUNDS AS PROTECTIVE OR THERAPEUTIC AGENTS AGAINST RADIATION INJURY IN EXPERIMENTAL ANIMALS

+ **I. The Influence of Various Chemical Compounds on Radiation Lethality in Mice**

V. Plzak, M. Root and J. Doull

This report concerns: The survival time and mortality of male CF_1 mice treated with various chemical compounds immediately prior to the administration of a lethal dose of whole-body x-irradiation.

Immediate or ultimate application of the results: To find chemical compounds capable of reducing or preventing mortality in x-irradiated animals and to elucidate some of the structure-activity relationships within groups of related chemical protective agents. Although none of the currently available radioprotective agents provide a practical solution to the problem of preventing acute radiation injury because of their toxicity or relative ineffectiveness, the study of these compounds and of related derivatives lacking protective or toxic effects provides the most logical approach for finding compounds with an improved chemotherapeutic ratio. A better understanding of the precise structural configuration(s) responsible for maximal radioprotective activity with minimal toxicity would also be of considerable aid in furthering our understanding of the basic mechanisms of radiation damage in biological systems.

* * * * * * * * * *

During the past three months forty-three additional chemical compounds representing several types of chemical structures related to known radioprotective agents have been evaluated for protective activity against lethality from whole-body x-ray exposure in mice (1).

Materials and Methods. Adult, male Carworth Farms (CF_1) mice weighing between 20 and 25 grams were employed for these studies. The animals were housed in air-conditioned rooms (75° F. to 80° F.) and were provided with food (Rockland Mouse Pellets) and water *ad libitum*. All of the animals were kept under observation for at least one week prior to their use during which time those mice which failed to gain weight normally or which appeared to be unhealthy were removed and sacrificed. Both the control and experimental animals were selected at random from a single shipment of animals in order that their age and weight would be comparable. Preliminary toxicity studies were carried out with each compound to determine the maximum amount of each derivative which could be administered to the mice without causing mortality due to chemical toxicity.

For the radiation studies a minimum of ten mice were tested at each dosage level. Distilled water was used as the solvent wherever possible and the concentration was adjusted in each case so that none of the mice received more than 2% of their body weight with each injection. The pH of the solutions was adjusted to approximately 7.0 when necessary using either dilute hydrochloric acid, sodium hydroxide or sodium bicarbonate. Compounds which were insoluble in water were dissolved in propylene glycol. Both the control mice (those treated with a comparable amount of injection vehicle) and the treated mice were x-rayed simultaneously 10 to 15 minutes following the intraperitoneal injections. Observations on the mortality of both groups were made daily for a period of 30 days after the exposure or until all of the mice in the treated group were dead.

The x-ray exposure was given as a single whole body exposure of 250 KVP, 15 ma. x-ray by means of either a G.E. Maximar Therapy Unit or Kelekat Therapy Unit. The dose rate was measured prior to each radiation period by means of a Victoreen Ionization Chamber (100 r thimble) and was found to be between 40 r and 42 r per minute. The added filtration consisted of 0.25 mm. of copper and 1.0 mm. of aluminum and the target skin distance was 75 cm. The animals were irradiated individually in plastic tubes (50 cc. centrifuge tubes provided with numerous holes for air) placed radially on a rotating turntable so that each animal received an equal dose of x-ray. The turntable was enclosed in a temperature-controlled chamber which maintains a temperature of approximately 76° F. to 78° F. during the irradiation exposure period.

The USAF code letter designation and the source of the compounds included in this study are listed in Table 1.

TABLE 1

SOURCE AND USAF CODE NUMBER OF COMPOUNDS INCLUDED IN THIS REPORT

USAF Designation	Source of Compound
FA	Fairmount Chemical Company, Inc., Newark, New Jersey
MJ	Dr. E. W. Durachta, Mead Johnson Research Ctr., Evansville, Ind.
EK	Eastman Kodak Company, Rochester, New York
K	Dr. G. Thiessen, Koppers Company, Inc., Pittsburgh, Pa.
CB	California Corp. for Biochemical Research, Los Angeles, Calif.
ST	Dr. N. W. Standish, The Standard Oil Company, Cleveland, Ohio
AN	Mr. M. L. Neuville, Ansul Chemical Company, Marinette, Wisc.
PD	Dr. R. D. Westland, Parke Davis and Company, Ann Arbor, Mich.

Results

Preliminary toxicity studies. In order to determine the maximum safe dose for use in the radiation studies, it was necessary to obtain an approximate LD_{50} for the various compounds. Accordingly, small groups of mice were injected intraperitoneally with increasing dosage levels of each compound, and the resulting mortality was recorded for a period of one week. The results of these toxicity tests are shown in Table 2.

Evaluation of compounds for radioprotective activity. The x-ray dose used for these studies (750 r of whole body x-irradiation delivered in a single dose) produces over 99% mortality in CF_1 mice under the experimental conditions used in this laboratory. Radiation deaths first appear on the fifth and sixth day after the exposure and the median survival time (ST_{50}) of control or untreated animals is 11 ± 3 days. A compound is considered to exhibit protective effects if it increases the ST_{50} by more than five days or if it permits any of the animals to survive for 30 days after the x-ray exposure.

The first seven compounds studied included a thiazole derivative, two thiadiazole derivatives and a tetrazole derivative. Of these the thiazole, 2,4-dimethylthiazole (FA-7) and one of the diazoles, 2-amino-5-mercapto-1,3,4-thiadiazole (FA-6) were protective. In Figure 1 it may be seen that when FA-7 was administered at a dosage level of 200 mgm./kgm., it protected 10% of the mice from an otherwise lethal dose of whole body x-irradiation, and that 2-amino-5-mercapto-1,3,4-thiadiazole (FA-6) protected 50% of the mice when given at the same dosage level. In the latter instance lowering the dose to 100 mgm./kgm. eradicated the protective effect. In a previous report (2) a marked radioprotective effect was obtained with 2-butanone oxime. Consequently alpha benzoin oxime was evaluated but no protection was apparent. The thiobenzophenone derivative tested at this time was also of no value but a thiourea (allylhydroxyethyl thiourea, FA-3) did protect 10% of the mice injected with 100 mgm./kgm. of this compound prior to a lethal x-ray exposure. Figure 2 depicts this graphically.

Two carbazoles, aminoethyl and diaminoethyl carbazole (K-3132 and K-3455 respectively) both showed protective activity against radiation lethality. In both instances the higher dosage level employed was toxic as evidenced by a decrease in the ST_{50} while a dosage level of 50 mgm./kgm. gave protection. Figure 2 shows 50% survival of mice pretreated with the monoaminoethyl carbazole and Figure 3 shows that only 10% of the mice similarly treated with the diaminoethyl carbazole survived for 30 days after 750 r.

Since thioglycerol showed radioprotective activity when tested previously (1), this compound and several related compounds were included in the present tests. However, no protection was afforded by any of these compounds at the dosage levels used. Minimal protection (10% survival of mice for 30 days following a lethal x-ray exposure) was obtained following the administration of 100 mgm./kgm. of dithiothymine (CB-38). These results are shown in Figure 3.

The next group of 14 compounds consisted mainly of butane derivatives. However, since the names and structures of those derivatives have not been released as yet, they are presented in this report by their code numbers only. Of these, ten showed varying amounts of radioprotective activity. Figures 4, 5, 6, 7 and 8 depict the results graphically.

TABLE 2

ACUTE INTRAPERITONEAL TOXICITY AND RADIOPROTECTIVE ACTIVITY OF VARIOUS CHEMICAL COMPOUNDS IN MALE CF_1 MICE

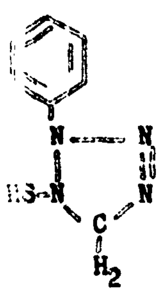
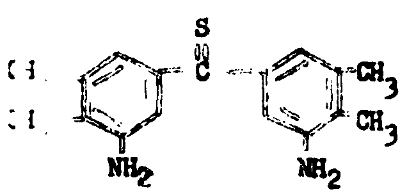
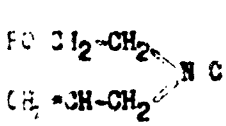
Name and Formula of Compound, USAF No. and Vehicle Used for Toxicity and Radiation Tests	Toxicity	Radiation Studies		
	Approx. LD_{50} in mgm./kgm.	Dose in mgm./kgm.	Change in ST_{50} in Days	Mortality at 30 Days After X-ray
1-Phenyl-5-mercaptotetrazole FA 1 (PG) 	200-300	200 50	-11 - 1	10/10 10/10
Tetramethyldiaminothiobenzophenone FA 2 (PG) 	200-300	200 100	- 3 - 1	10/10 10/10
Allyl hydroxyethylthiourea FA 3 (PG) 	>1000	1000 500	0 - 1	9/10 10/10

TABLE 2--Continued

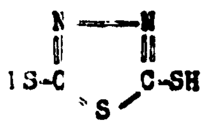
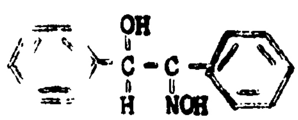
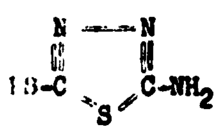
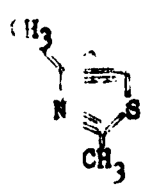
Name and Formula of Compound, USAF No. and Vehicle Used for Toxicity and Radiation Tests	Toxicity	Radiation Studies		
	Approx. LD_{50} in mgm./kgm.	Dose in mgm./kgm.	Change in ST_{50} in Days	Mortality at 30 Days After X-ray
2,5-Dimercapto-1,3,4-thiadiazole FA-4 (H_2O plus heat) (same as A-8354) 	500-1000	200 100	- 5 - 3	10/10 10/10
Alpha benzoin oxime FA-5 (PG) 	100-200	100 50	- 5 0	10/10 10/10
2-Amino-5-mercapto-1,3,4-thiadiazole FA-6 (PG) 	200-300	200 100	+ 2 - 2	5/10 10/10
2,4-Dimethylthiazole FA-7 (PG) 	200-300	200 100	0 0	9/10 10/10

TABLE 2--Continued

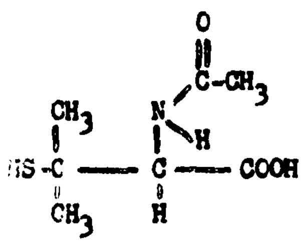
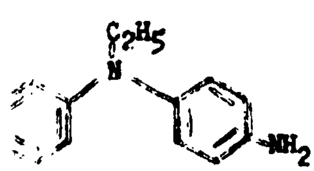
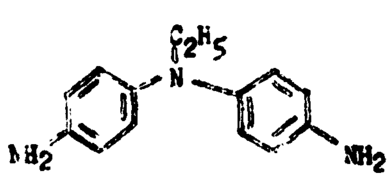
Name and Formula of Compound, USAF No. and Vehicle Used for Toxicity and Radiation Tests	Toxicity	Radiation Studies		
	Approx. LD_{50} in mgm./kgm.	Dose in mgm./kgm.	Change in ST_{50} in Days	Mortality at 30 Days After X-ray
N-Acetyl-D,L-penicillamine (5179) MJ-2 (PO) 	300-500	300 200	- 4 + 1	10/10 10/10
,N'-Dithiodiformamidine di-hydrochloride K-8479 (H_2O) 	200	100 50	- 4 + 3	10/10 10/10
-Amino-9-ethyl carbazole -3132 (PO) 	100-200	100 50	-10 + 1	10/10 5/10
,6-Diamino-9-ethyl carbazole -[illegible]455 (PO) 	100-200	100 50	- 2 0	10/10 9/10

TABLE 2--Continued

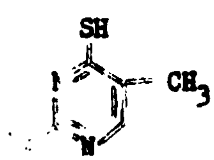
Name and Formula of Compound, USAF No. and Vehicle Used for Toxicity and Radiation Tests	Toxicity	Radiation Studies		
	Approx. LD_{50} in mgm./kgm.	Dose in mgm./kgm.	Change in ST_{50} in Days	Mortality at 30 Days After X-ray
Thioglycolic acid--98% CB-35 (H_2O) $HS-CH_2COOH$	100-200	100 50	- 5 - 1	10/10 10/10
Thiodiglycolic acid--98% CB-[illegible]5 (H_2O) (also E-2) CH_2COOH CH_2COOH	200-300	200 100	- 9 0	10/10 10/10
[illegible]hioglycerol [illegible]B-[illegible]7 (H_2O) (same as B-40) C-SH C-OH C-OH	300-500	300 100	0 + 1	10/10 10/10
[illegible]iothymine [illegible]-[illegible]3 (PG) 	200	100 50	0 + 1	9/10 10/10
[illegible]T-[illegible] (20923)	200-300	50 25	+10 + 1	6/10 10/10

TABLE 2--Continued

Name and Formula of Compound, USAF No. and Vehicle Used for Toxicity and Radiation Tests	Toxicity	Radiation Studies		
	Approx. LD_{50} in mgm./kgm.	Dose in mgm./kgm.	Change in ST_{50} in Days	Mortality at 30 Days After X-ray
ST-2 (209214)	>1000	1000 500	+ 5 - 2	6/10 10/10
ST-3 (20926)	300-500	300 200	0 - 1	9/10 10/10
ST-4 (20927)	200-300	200 100	0 + 2	10/10 10/10
ST-5 (20928)	>1000	1000 500	+ 1 0	10/10 10/10
ST-6 (20929)	50-100	50 25	0 - 3	9/10 10/10
ST-7 (209211)	>1000	1000 500	0 0	7/10 10/10
ST-8 (209215)	>1000	1000 500	+ 4 + 1	5/10 6/10
ST-9 (209227)	>1000	1000 500	- 1 0	6/10 5/10
ST-10 (209230)	200-300	200 100	- 1 + 2	10/10 10/10
ST-11 (209240)	300-500	300 100	+ 2 + 3	10/10 10/10
ST-12 (209247)	300-500	100 50	0 0	10/10 9/10

TABLE 2--Continued

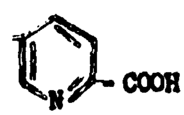
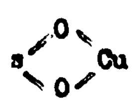
Name and Formula of Compound, USAF No. and Vehicle Used for Toxicity and Radiation Tests	Toxicity	Radiation Studies		
	Approx. LD_{50} in mgm./kgm.	Dose in mgm./kgm.	Change in ST_{50} in Days	Mortality at 30 Days After X-ray
ST-33 (209253)		75 50	+ 1 + 3	10/10 10/10
ST-34 (209254)	200-300	200 100	>18 + 1	4/10 7/10
Dimercurous methane arsonate N- (H_2O) C- sO_3Hg_2	50-100	50 10	-11 - 7	10/10 10/10
inchomeronic acid (pyridine 5-dicarboxylic acid) N- (H_2O plus $NaHCO_3$) CO 	>1000	1000 500	- 6 0	10/10 10/10
op er methane arsonate N- (H_2O plus $NaHCO_3$) C- 	10-25	10 5	- 6 + 2	10/10 9/10
dylic acid (H_2O) H_3 As-O-H (=O) H_3	500-1000	500 200	- 3 + 4	10/10 6/10

TABLE 2--Continued

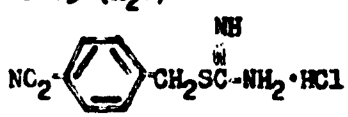
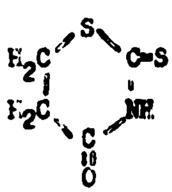
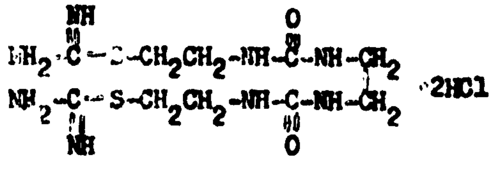
Name and Formula of Compound, USAF No. and Vehicle Used for Toxicity and Radiation Tests	Toxicity	Radiation Studies		
	Approx. LD_{50} in mgm./kgm.	Dose in mgm./kgm.	Change in ST_{50} in Days	Mortality at 30 Days After X-ray
N-Butylene pyrrolidine AN-6 (H_2O) 	50	25 10	- 2 + 3	10/10 9/10
2-(p-Nitrobenzyl)-2-thio-pseudourea monohydrochloride PD-23 (H_2O) 	50-100	50 25	+ 1 - 4	10/10 10/10
2-Thio-m-thiazone-2,4-dione PD-24 (H_2O) 	500-1000	400 200	- 1 - 4	10/10 10/10
1,1'-Ethylene bis-{3-[2-(amidino-thio)ethyl] urea} dihydrochloride PD-34 (PG) 	100	50 25	+ 2 - 2	10/10 10/10

TABLE 2--Continued

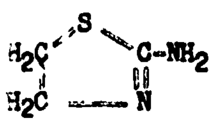
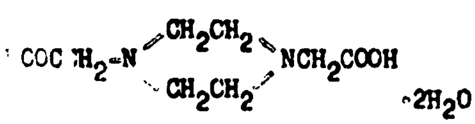
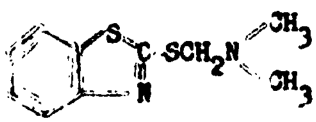
Name and Formula of Compound, USAF No. and Vehicle Used for Toxicity and Radiation Tests	Toxicity	Radiation Studies		
	Approx. LD_{50} in mgm./kgm.	Dose in mgm./kgm.	Change in ST_{50} in Days	Mortality at 30 Days After X-ray
2-Amino-2-thiazoline PD-57 (PG) 	100-200	100 50	- 1 - 5	10/10 10/10
5-Chloro-2-mercapto benzothiazole PD-59 (PG) 	100-200	100 50	+ 2 - 5	10/10 10/10
Piperazine diacetic acid, dihydrate [illegible] (PG) 	≥1000	500 300	- 4 - 3	10/10 10/10
2 (1-Pyrrolidinyl)ethyl-2-thiopseudourea dihydrochloride I-6 (H_2O) $\cdot CH_2-S-C(=NH)-NH_2 \cdot 2HCl$	100-200	100 50	- 2 - 1	10/10 10/10

TABLE 2--Continued

Name and Formula of Compound, USAF No. and Vehicle Used for Toxicity and Radiation Tests	Toxicity	Radiation Studies		
	Approx. LD_{50} in mgm./kgm.	Dose in mgm./kgm.	Change in ST_{50} in Days	Mortality at 30 Days After X-ray
β-(2-Aminoethylthio)propionic acid PD-62 (H_2O) $H_2NCH_2CH_2SCH_2CH_2COOH$	≫1000	500 300	- 5 - 5	10/10 10/10
2-(Dimethylaminomethylthio)-benzothiazole PD-65 (H_2O) 	100-200	100 50	+ 1 - 6	10/10 10/10

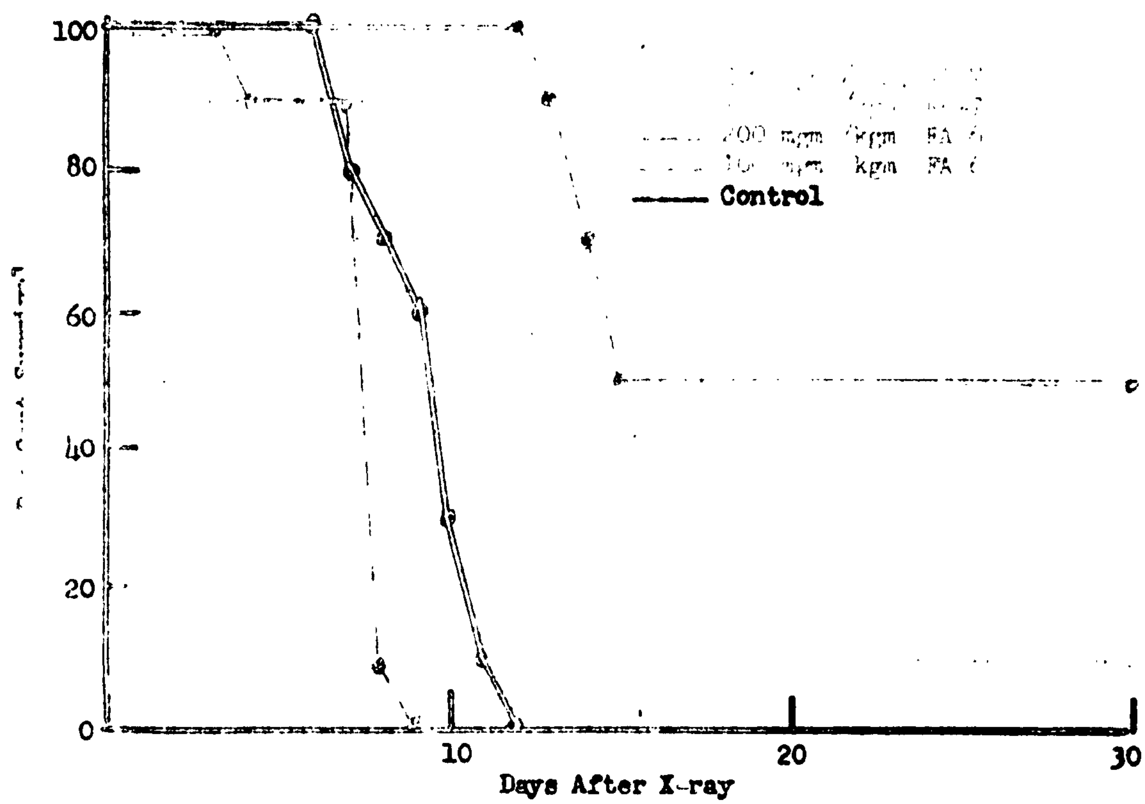


Figure 1. Effect of 2,4-dimethylthiazole (FA-7) and 2-amino-5-mercapto-1,3,4-thiadiazole (FA-6) on survival of mice irradiated with 750 r of whole body x-irradiation.

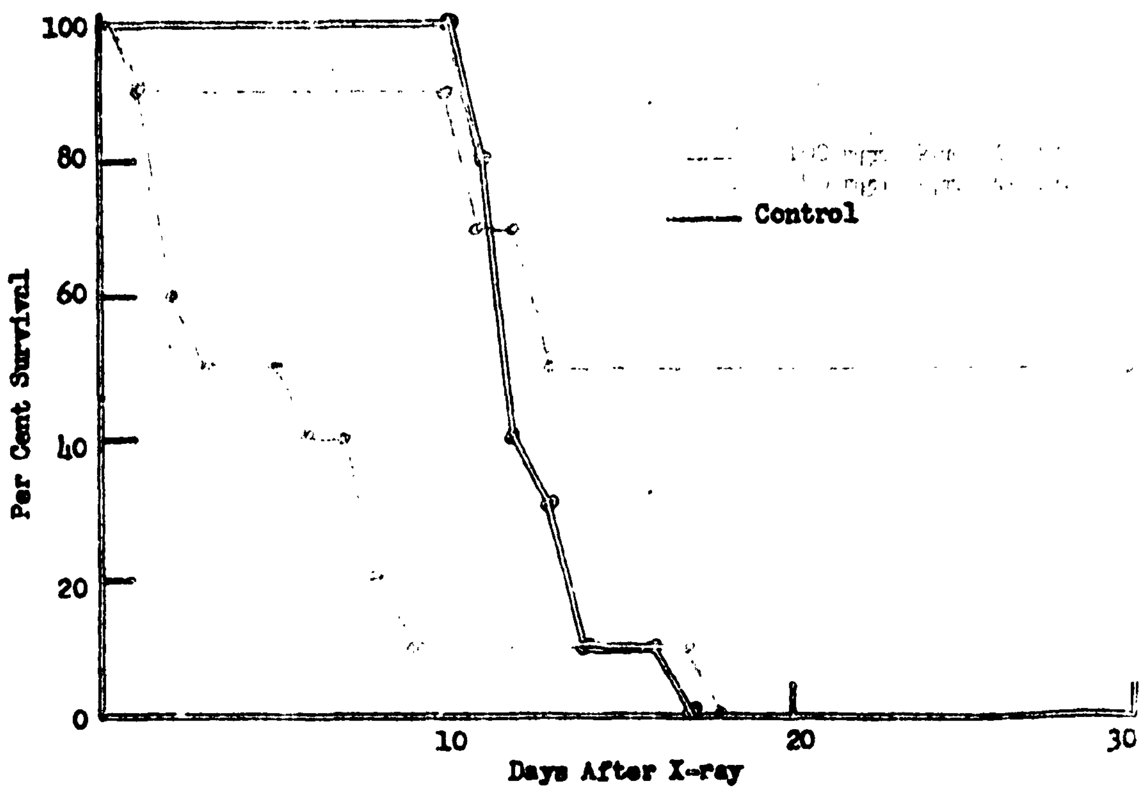


Figure 2. Effect of allyl hydroxyethylthiourea (FA-3) and 3-amino-9-ethyl carbazole (K-3132) on survival of mice irradiated with 750 r of whole body x-irradiation

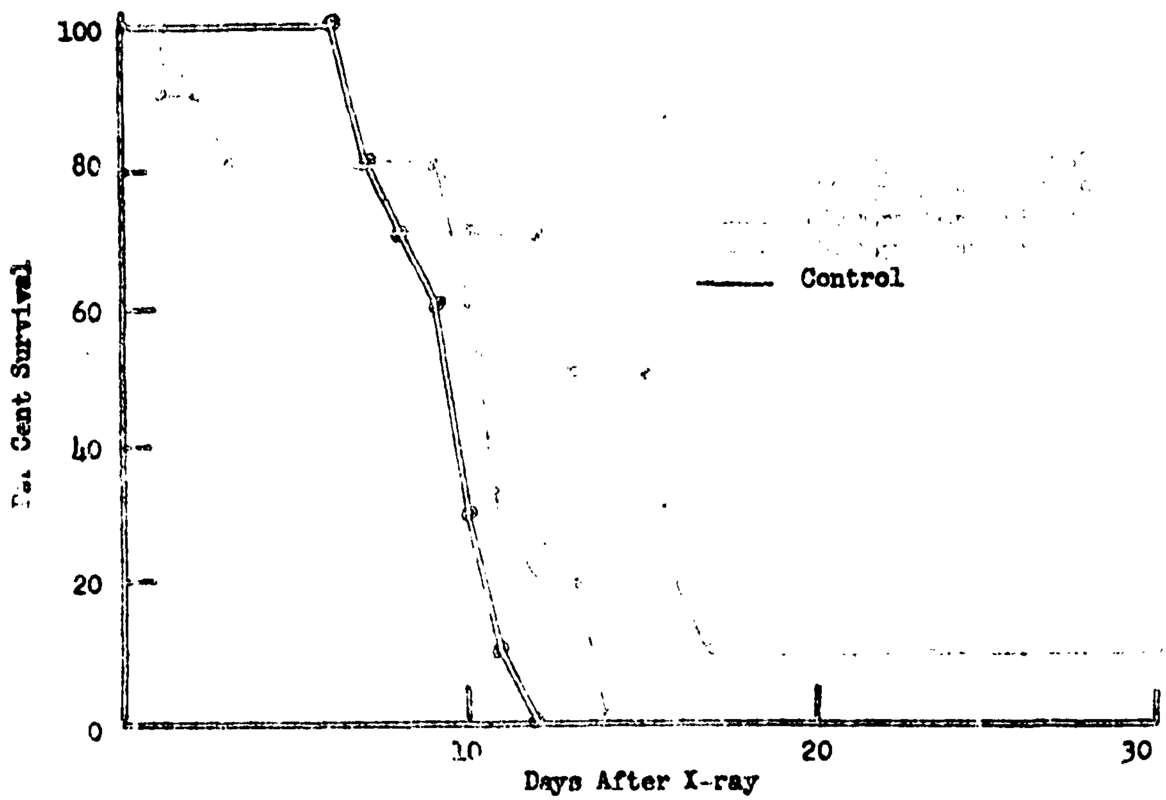


Figure 3. Effect of 3,6-diamino-9-ethyl carbazole (K-3455) and dithiothymine (CB-38) on survival of mice irradiated with 750 r of whole body x-irradiation.

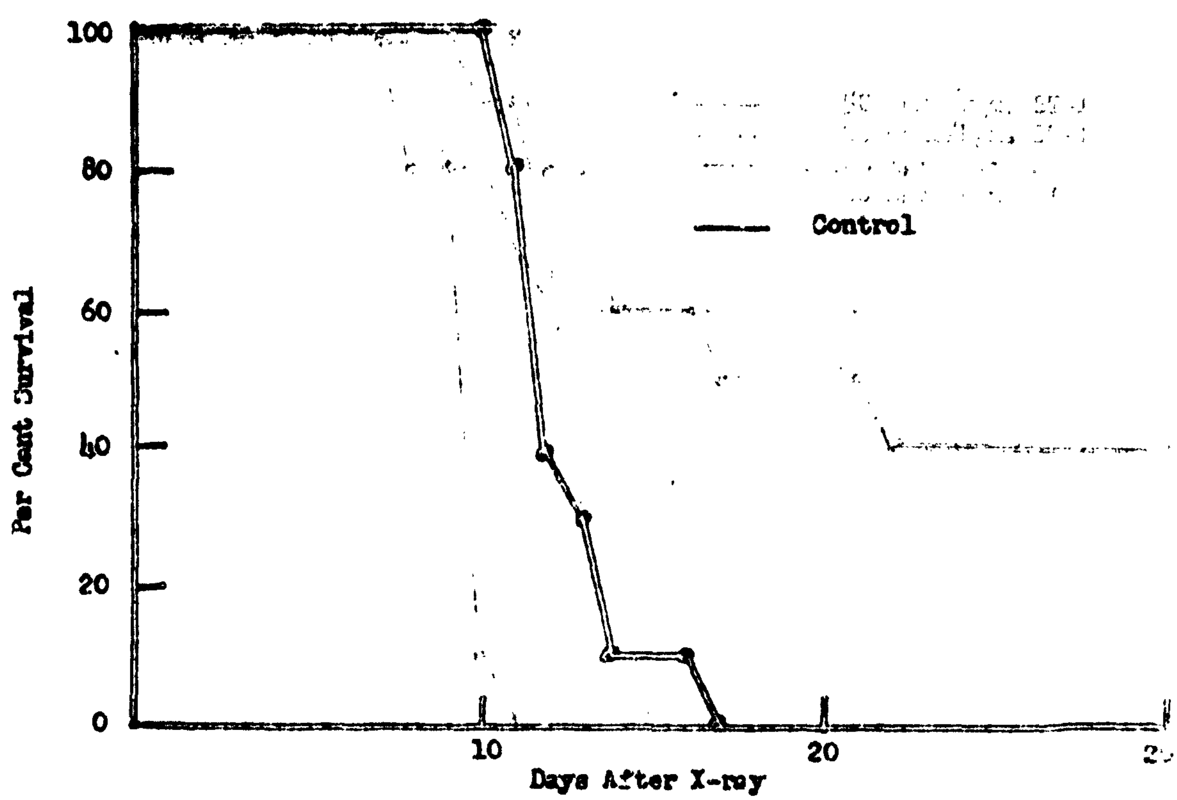


Figure 4. Effect of ST-1 and ST-2 on survival of mice irradiated with 750 r of whole body x-irradiation.

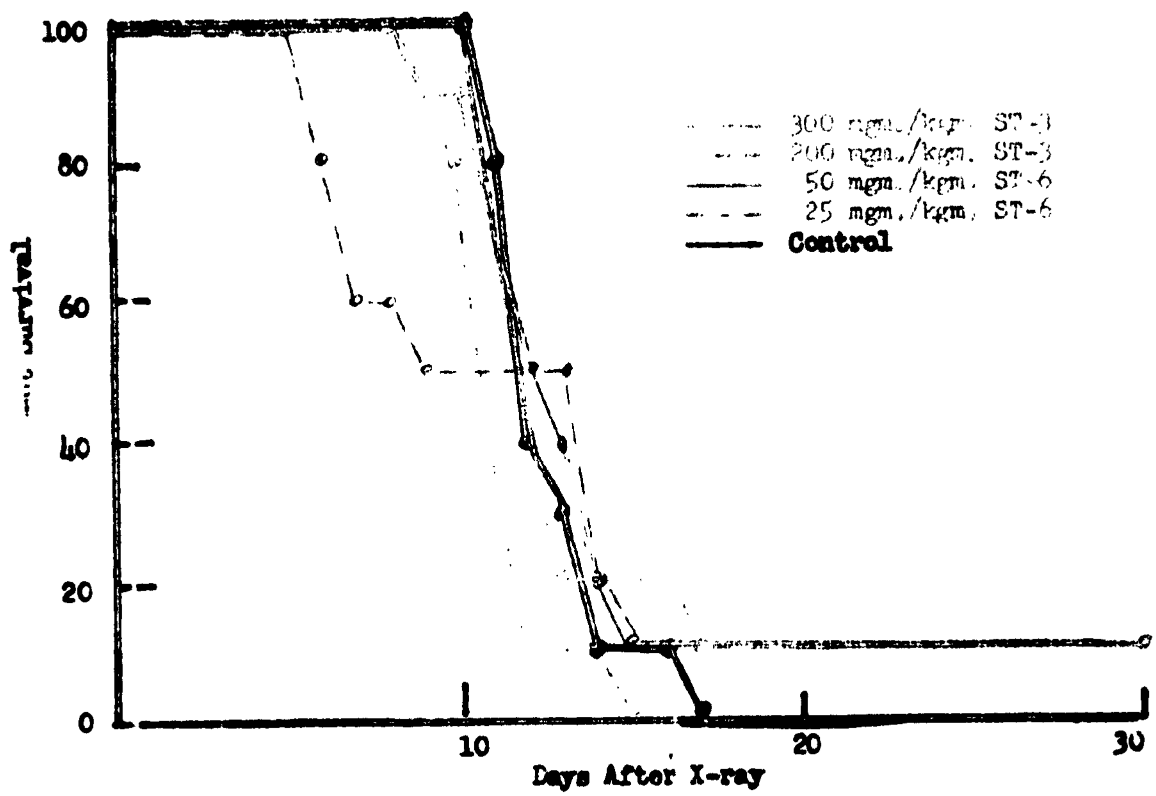


Figure 5. Effect of ST-3 and ST-6 on survival of mice irradiated with 750 r of whole body x-irradiation.

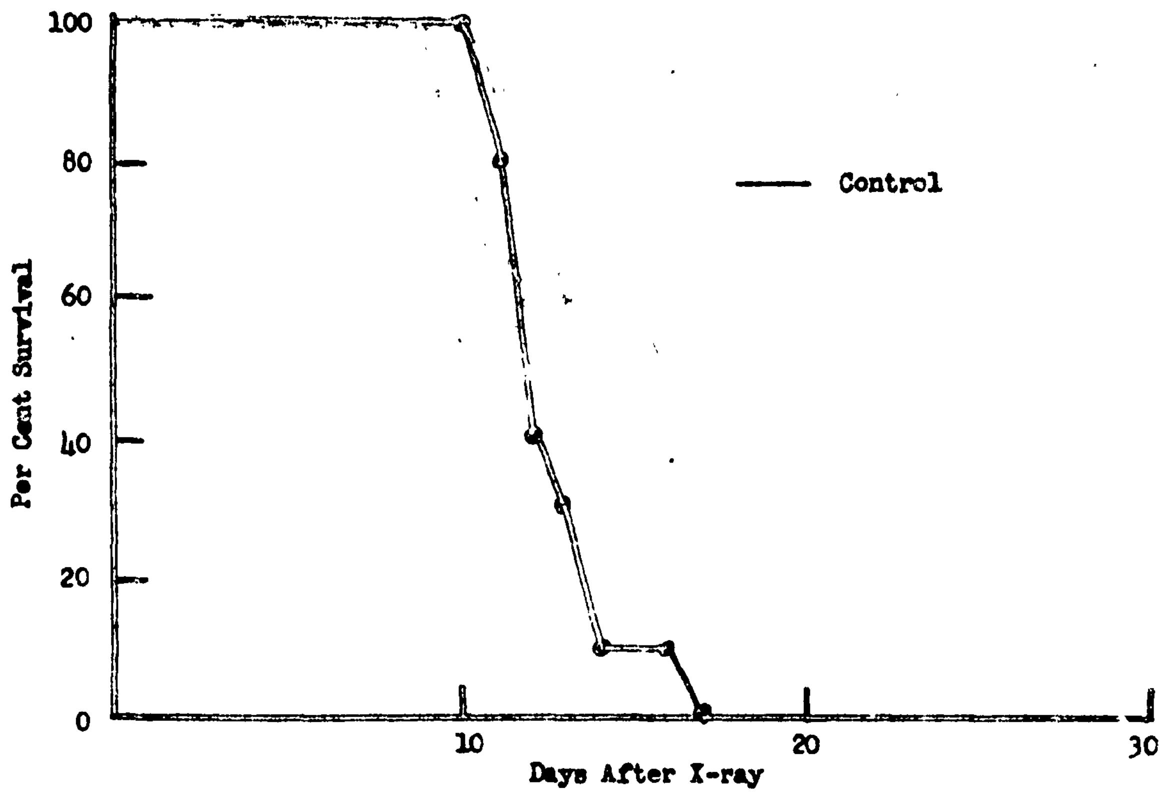


Figure 6. Effect of ST-7 and ST-8 on survival of mice irradiated with 750 r of whole body x-irradiation.

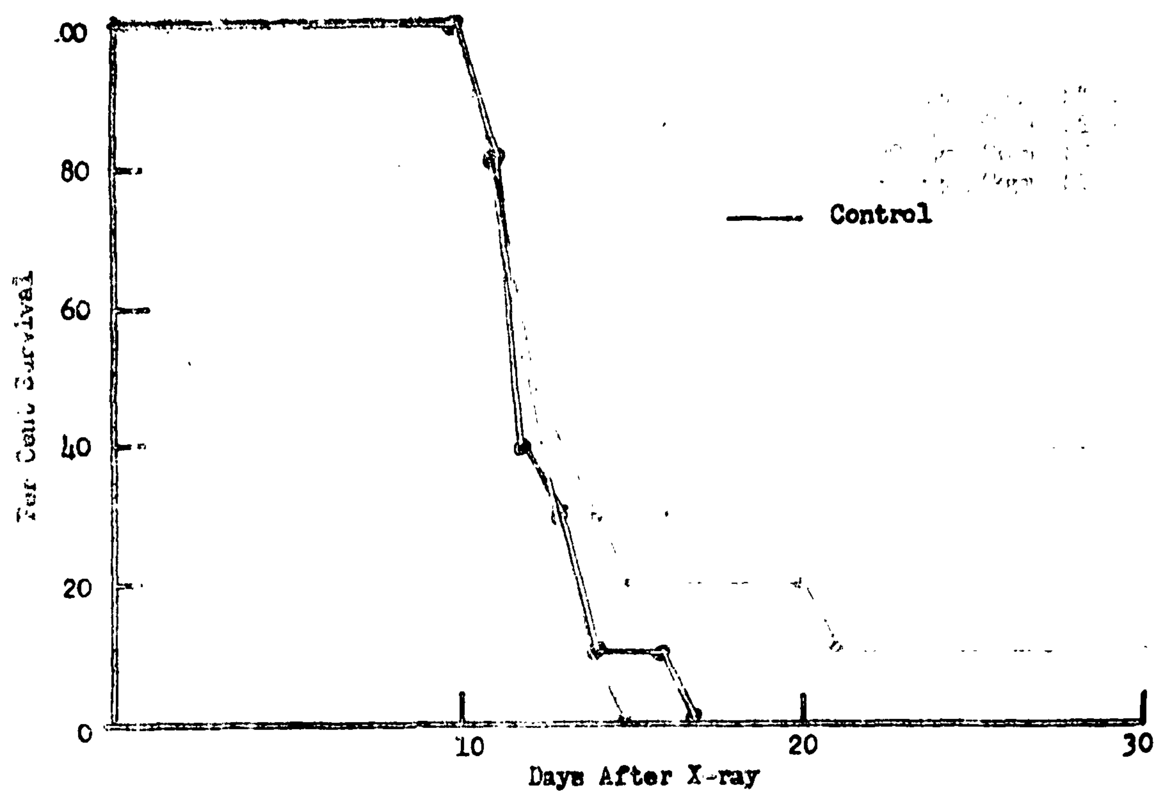


Figure 7. Effect of ST-9 and ST-12 on survival of mice irradiated with 750 r of whole body x-irradiation.

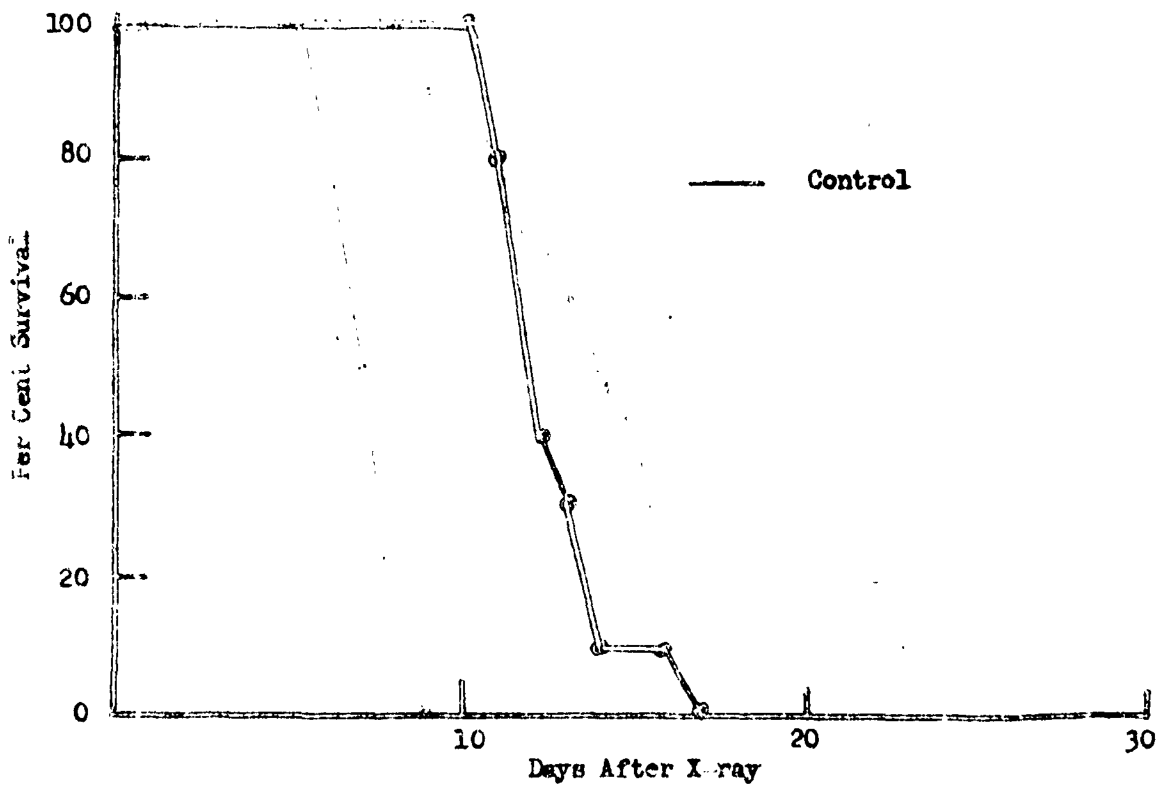


Figure 8. Effect of ST-14 and copper methane arsonate (AN-3) on survival of mice irradiated with 750 r of whole body x-irradiation.

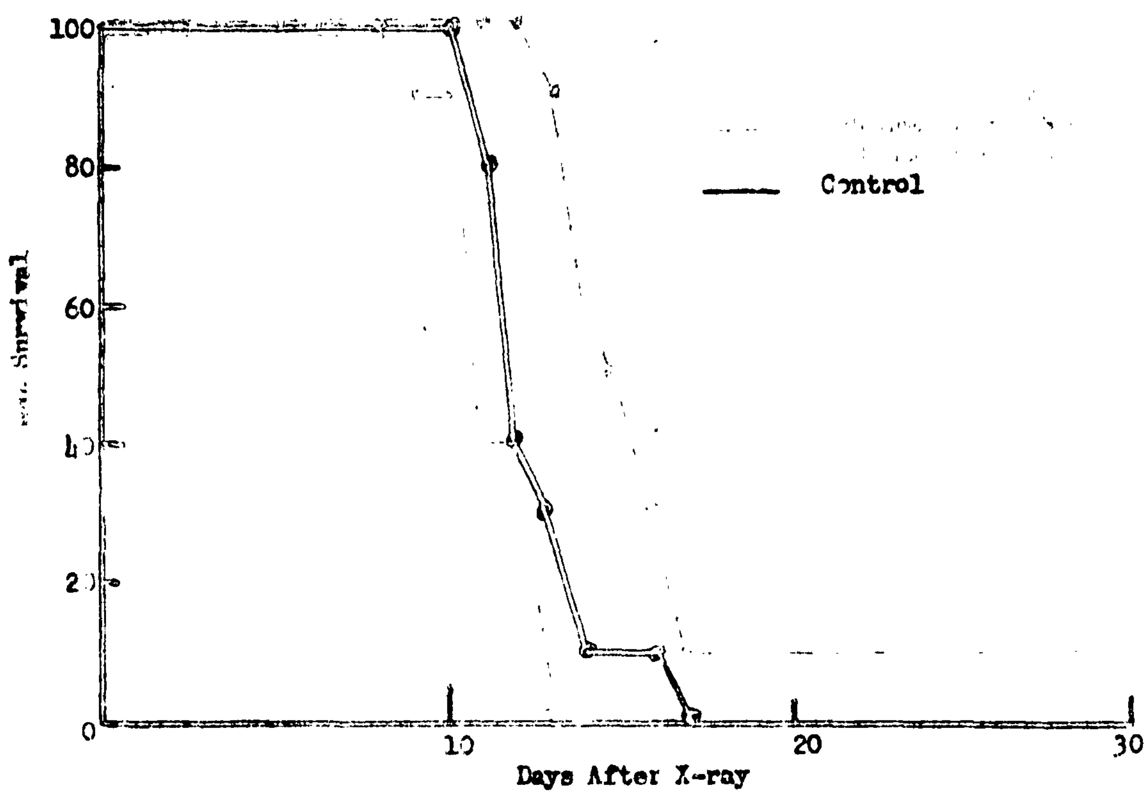


Figure 9. Effect of cacodylic acid (AN-5) and N-butylene pyrrolidine (AN-6) on survival of mice irradiated with 750 r of whole body X-irradiation.

Two arsonates, a mercurous and a copper derivative were evaluated and the latter (copper methane arsonate, AN-3) showed minimal protection as evidenced by a 10% survival of mice pretreated with 5 mgm./kgm. of this compound prior to a lethal x-ray exposure (Figure 8). Another arsenical, cacodylic acid (AN-5), similarly protected 40% of the mice when administered at a dosage level of 200 mgm./kgm. The higher dose of 500 mgm./kgm. not only resulted in no 30-day survivors but decreased the ST_{50} by three days from that of the control mice x-irradiated simultaneously. Figure 9 shows these results as well as those obtained when N-butylene pyrrolidine (AN-6) was administered to mice at a dosage level of 10 mgm./kgm. prior to a lethal exposure of 750 r of whole body x-irradiation.

The nine remaining compounds which contained ureas and pseudoureas, benzothiazoles, a piperazine, a thiazoline, a dione, and a propionic acid derivative were all ineffective as radioprotective compounds.

Summary

Forty-three chemical compounds have been tested for evidence of radioprotective activity in the present study. Of these, 18 showed slight to significant protective effects in that they permitted from 10% to 60% of the mice to survive for at least 30 days following the otherwise lethal whole body x-ray exposure. The maximum amount of protection was afforded when ST-14 was administered (60% and 30% respectively for the two doses tested). Four compounds (2-amino-5-mercapto-1,3,4-thiadiazole (FA-6), 3-amino-9-ethyl carbazole (K-3132), ST-8 and ST-9) protected 50% of the x-irradiated mice for 30 days following exposure whereas a 40% survival resulted from the pre-irradiation administration of three of the compounds included in this study. These compounds were: ST-1, ST-2 and cacodylic acid (AN-5). ST-7 protected 30% of the irradiated mice during the 30-day post-irradiation period. The remaining nine protective derivatives gave only minimal protection (10%, 30-day survivors); this group included: allyl hydroxyethyl thiourea (FA-3), 2,4-dimethylthiazole (FA-7), 3,6-diamino-9-ethyl carbazole (K-3455), dithiothymine (CB-38), ST-3, ST-6, ST-12, copper methane arsonate (AN-3), and N-butylene pyrrolidine (AN-6).

References

1. Doull, J., Plzak, V., and Brois, S. J., USAF Radiation Lab. Screening Program Status Report No. 2, August 1, 1961.

2. Plzak, V., Root, M., and Doull, J., USAF Radiation Lab Quarterly Progress Report No. 43, April 15, 1962, p. 16.

PHARMACOLOGICAL AND TOXICOLOGICAL COMPOUNDS AS PROTECTIVE OR THERAPEUTIC AGENTS AGAINST RADIATION INJURY IN EXPERIMENTAL ANIMALS

II. Further Studies on the Mechanism of Radioprotection Afforded by Cyanide and Various Nitriles

J. Dilley and J. Doull

This report concerns: The survival time and mortality of male and female CF_1 mice treated with cyanide, acetone cyanohydrin, pentanone cyanohydrin and 2-imino-thiazolidine-4-carboxylic acid prior to or following the administration of lethal doses of whole body x-irradiation, and some preliminary studies on the enzyme system involved in the detoxification of cyanide.

Immediate or ultimate application of the results: To obtain information concerning the mechanism(s) responsible for the radioprotective effects of cyanide and related derivatives. These studies constitute part of a program designed to obtain information regarding both the toxic and the protective effects of the currently available radioprotective agents. This information is essential for the evaluation of the potentially useful agents and the development of new agents or combinations of agents having an improved therapeutic index.

* * * * * * * * *

In a previous report (1) we described experiments which demonstrated that cyanide and some nitriles will protect mice against lethal doses of whole body x-irradiation. The present report is concerned with further studies on cyanide and some related cyanide-containing compounds, and the enzymes involved in the detoxification of cyanide. These studies suggest that these enzymes may be intimately involved in the radioprotective effects of such agents.

Materials and Methods. Adult, male and female Carworth Farms CF_1 mice weighing between 20 and 25 grams were used for these studies. The control and experimental animals were selected from single shipments, housed in groups of not more than ten animals per cage in an air-conditioned room (75° to 85° F.), and fed Rockland Mouse Pellets and water ad libitum. Aqueous solutions of the compounds used for these studies were prepared just prior to their use, and were injected intraperitoneally at a concentration of 1% or [illegible]s of the body weight.

The enzyme used for these studies was prepared from fresh rat liver according to the method of Cosby and Sumner (2). All extraction procedures were carried out at 4° C. and the preparations were stored at the same temperature in the proper dilutions of buffer.

The x-ray exposures were given by means of a G. E. Maximar X-ray Unit operated at 250 KVP and 15 ma. with 1.0 mm. of aluminum and 0.25 mm. of copper added filtration. The dose rate was determined prior to each irradiation exposure by means of a 250 r Victoreen Ionization Thimble in air. All of these studies were carried out at a dose rate of about 10 r per second to insure that the irradiation period would be as short as possible. Both the control and the treated animals were irradiated simultaneously and the mortality within each group was followed for 30 days or until the death of all of the mice in each group had occurred.

Results

Effect of increasing doses of cyanide on the survival time and mortality of x-irradiated female CF_1 mice. It has previously been shown (1) that cyanide is a good protector against lethal doses of ionizing radiations when given in doses of 2 mgm./kgm. as HCN just prior to the irradiation period. It was, therefore, of interest to see if a greater increase in the cyanide dose would afford even better protection with increasing doses of irradiation.

Groups of animals, each of which contained eight female mice, were used for these studies. Each group of animals was injected with either 2 or 2.25 mgm./kgm. of HCN (4.8 or 5.5 mgm./kgm. of the potassium salt). Approximately four minutes later these animals were then exposed to increasing amounts of whole body x-irradiation at dosage levels ranging from 700 r to 900 r in 50 r increments. The dose rate for these studies was about 230 r per minute and the results are shown in Figures 1 and 2. It can be seen that under these experimental conditions the higher dose of cyanide is more radioprotective. Unfortunately, higher doses of x-ray were not given since the observed response was greater than had been anticipated. These studies have been repeated at these dose levels and also at higher levels of both cyanide and irradiation but the results are not yet available.

Effect of acetone cyanohydrin and pentanone cyanohydrin on the mortality and survival time of female mice. In a previous report (1) we showed that acetone cyanohydrin is a good protective agent when given just prior to lethal doses of x-irradiation. We have since repeated this experiment using additional time intervals and compared this with pentanone cyanohydrin, which is less toxic than either cyanide or acetone cyanohydrin, but would be expected to be a good protector by virtue of the fact that it will also lose its cyanide radical quite rapidly in vivo.

Solutions of these two compounds were prepared by making the original dilution in the ratio of 10:1 (v/v) in propylene glycol and the final dilution was made (w/v) in distilled water. Groups of animals, each of which contained eight female CF_1 mice, were injected with these compounds at various time intervals prior to 700 r of whole body x-ray. Control animals were injected with a water placebo which contained about the same amount of propylene glycol as was injected into the experimental groups. The survival of these animals is shown in Figures 3 and 4. For comparative purposes the data at 2, 5 and 10 minutes for acetone cyanohydrin is included in Figure 3, although it has been previously reported (1). The data in these figures show that both of these compounds are

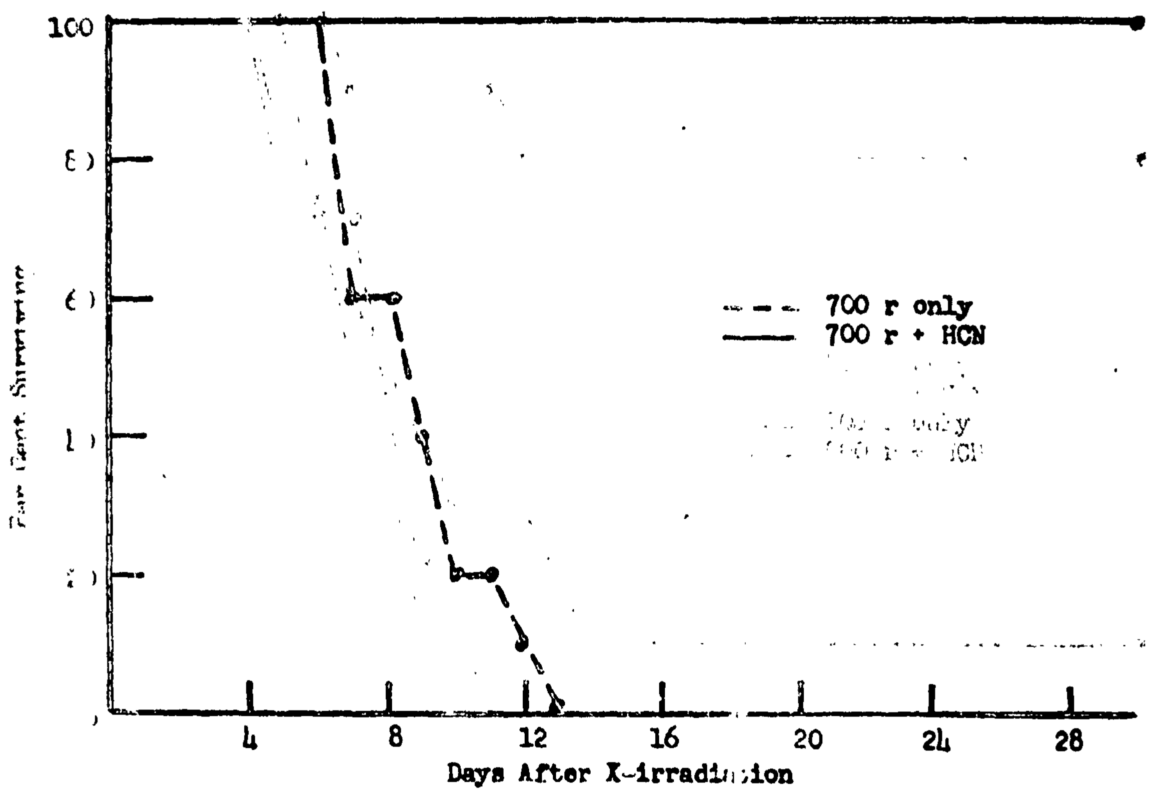


Figure 1. Effect of pre-treatment with 2.0 mgm. HCN/kgm. on the survival time and mortality of CF_1 female mice exposed to whole body x irradiation.

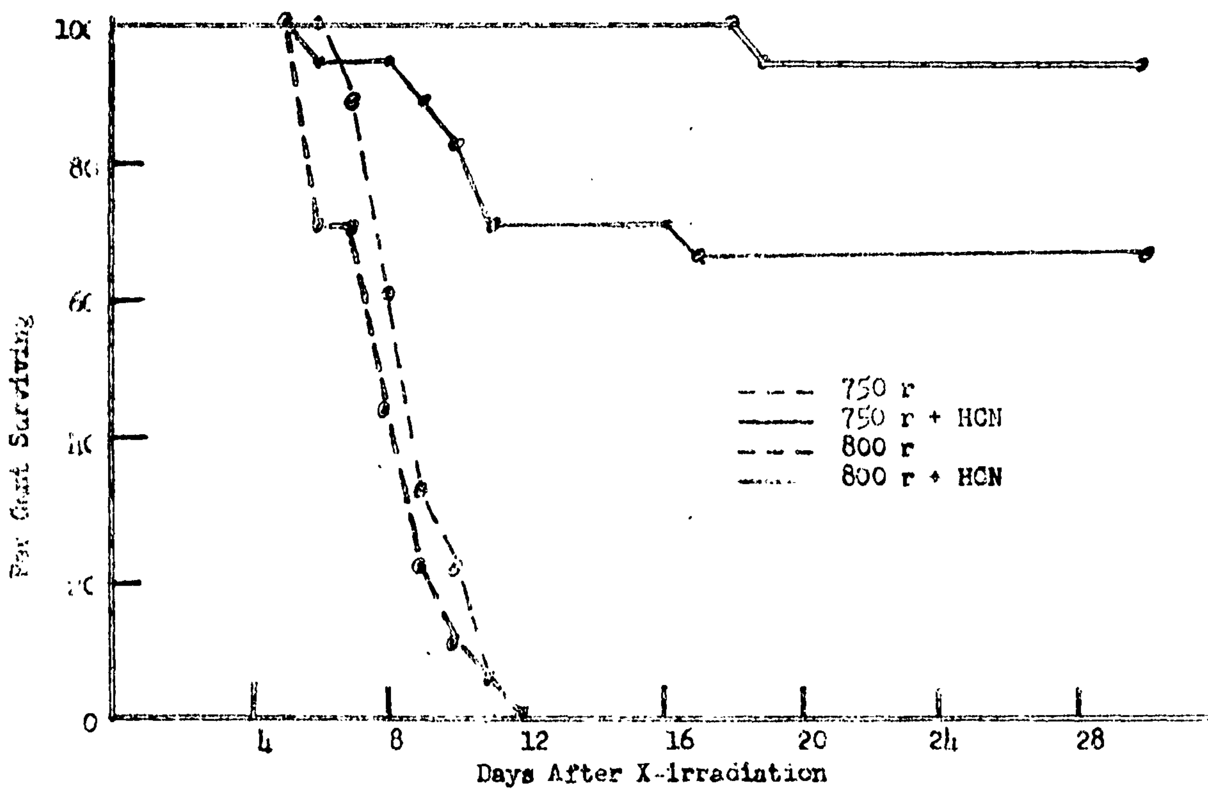


Figure 2. Effect of pre treatment with 2.25 mgm./HCN kgm. on the survival time and mortality of CF_1 female mice exposed to whole body x-irradiation

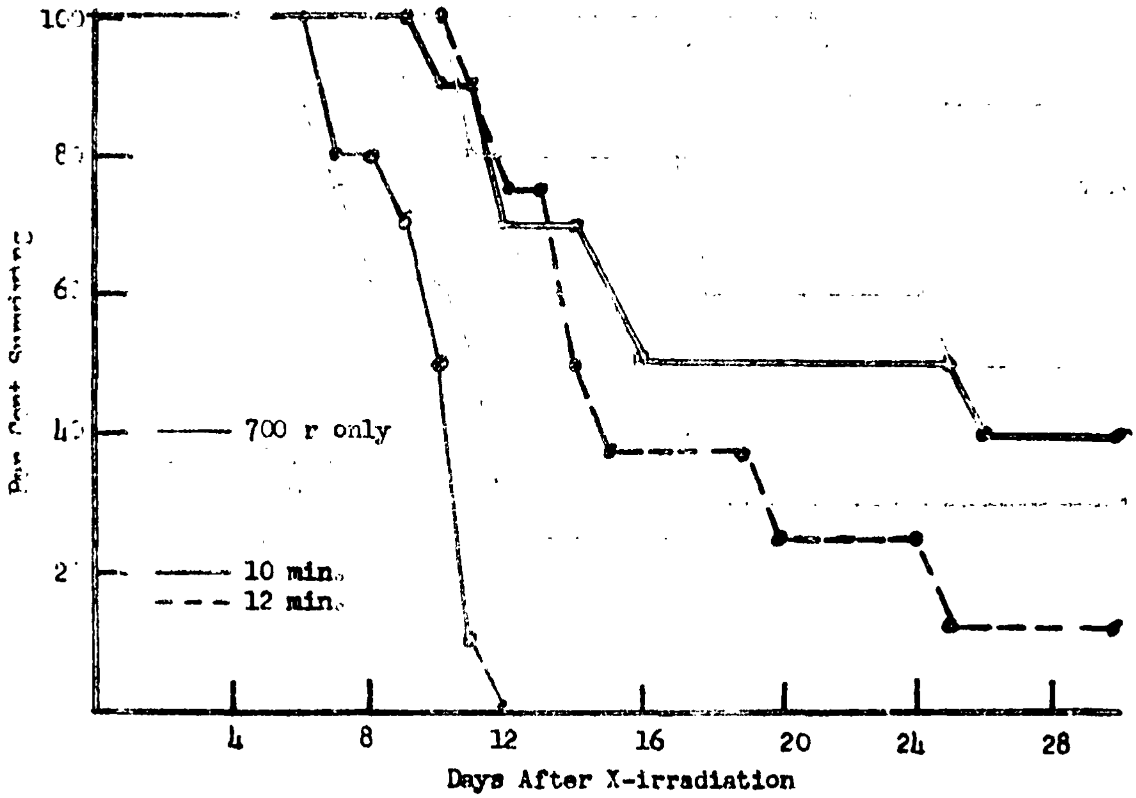


Figure 3. Survival time and mortality in CF_1 female mice given .3 mgm./kgm. of acetone cyanohydrin at various intervals prior to the administration of 700 r of whole body x-irradiation.

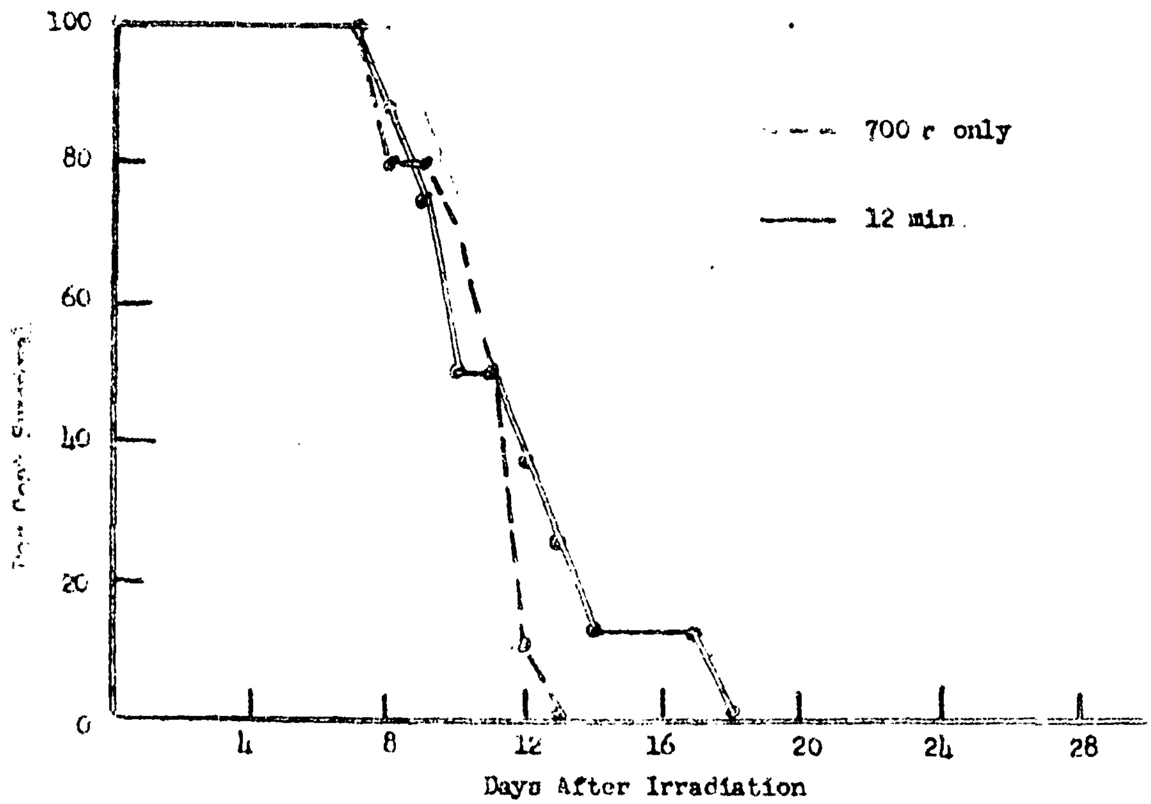


Figure 4. Survival time and mortality in CF_1 female mice given 10 mgm./kgm. of pentanone cyanohydrin at various intervals prior to 700 r of whole body x-irradiation.

good protective agents under these experimental conditions. Although the dose of pentanone cyanohydrin will have to be increased to nearer its toxic limits, the results of this experiment seem to indicate that the maximum protection with pentanone cyanohydrin occurs at about eight minutes while the maximum effect with acetone cyanohydrin occurs at four minutes. Further studies are planned to investigate the structure-activity relationships with these and other cyanohydrins.

<u>The radioprotective effect of 2-imino-thiazolidine-4-carboxylic acid in female CF_1 mice.</u> In a previous report (I) 2-imino-thiazolidine-4-carboxylic acid, a metabolite of cyanide, was discussed as a possible protective agent which might be responsible for a delayed protection observed with cyanide.

Mauthner (3) first described the reaction of cyanide with l-(+)-cystine in aqueous solution to form 2-imino-thiazolidine-4-carboxylic acid and this method was used to prepare the thiazolidine for these studies except that approximately equimolar amounts of cyanide and cystine were mixed together and the compound was then injected without further purification. Just prior to use the pH of the preparation was adjusted to about 7.6 with 1 N HCl. The reaction was considered to be complete when doses equivalent to 30-40 lethal doses of cyanide did not produce mortality in mice.

Groups of animals, each of which contained ten female mice, were used for these studies. The experimental animals were injected intraperitoneally at varying time periods prior to an exposure to 700 r of whole body x-irradiation. The control animals were injected with water. The dose of thiazolidine given each group was equal to 50 mgm./kgm. of HCN. In addition, two more groups were injected with doses of thiazolidine equal to 150 mgm./kgm. of HCN 5 and 30 minutes prior to 700 r of whole body x-irradiation. The survival curves for these animals are shown in Figure 5. It appears that under these experimental conditions, thiazolidine affords the best radioprotective effect when given 30 minutes prior to 700 r and that the higher dose of thiazolidine is more effective in preventing radiation lethality. However, at each dose level of the compound, and at every time interval, there was a substantial increase in the median survival time of the treated mice even though all of the groups did not exhibit 30-day survivors.

<u>The radioprotective effects of 2-imino-thiazolidine-4-carboxylic acid when given after exposure to lethal doses of whole body x-irradiation.</u> Because of the time effect relationships, it was thought worthwhile to investigate the possibility of obtaining protection with thiazolidine given after the radiation exposure. For this experiment four groups of ten mice were exposed to 700 r whole body x-ray at a dose rate of 10 r per second. Three of these groups were then injected with thiazolidine at [illegible], 15 or 30 minutes after the radiation exposure. The fourth group was injected with only water at 30 minutes after irradiation. The results of this experiment can be seen in Figure 6. While none of the animals survived the 30 day post exposure period, there was a prolongation of median survival time in the group treated at 30 minutes after irradiation. [illegible] therefore, to repeat the experiment using a lower dose level of radiation. In repeating this experiment, three groups of ten animals each were exposed to 650 r of whole body x-irradiation. The groups were then injected with thiazolidine equivalent to 150 mgm./kgm. of HCN at intervals of 15 [illegible] minutes. The third control group was again injected with water. The

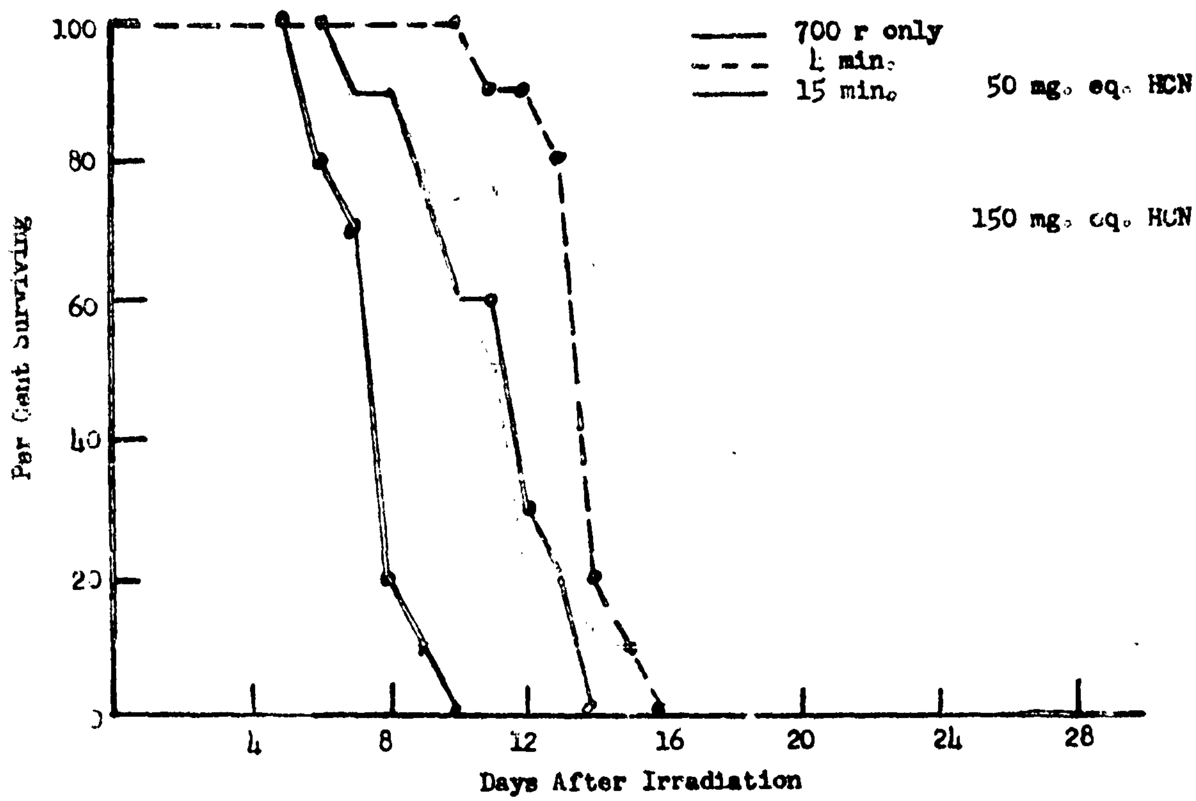


Figure 5. The effect of pre-irradiation administration of 2-imino-thiazolidine-4-carboxylic acid on the survival time and mortality of CF_1 female mice exposed to 700 r of whole body x-irradiation.

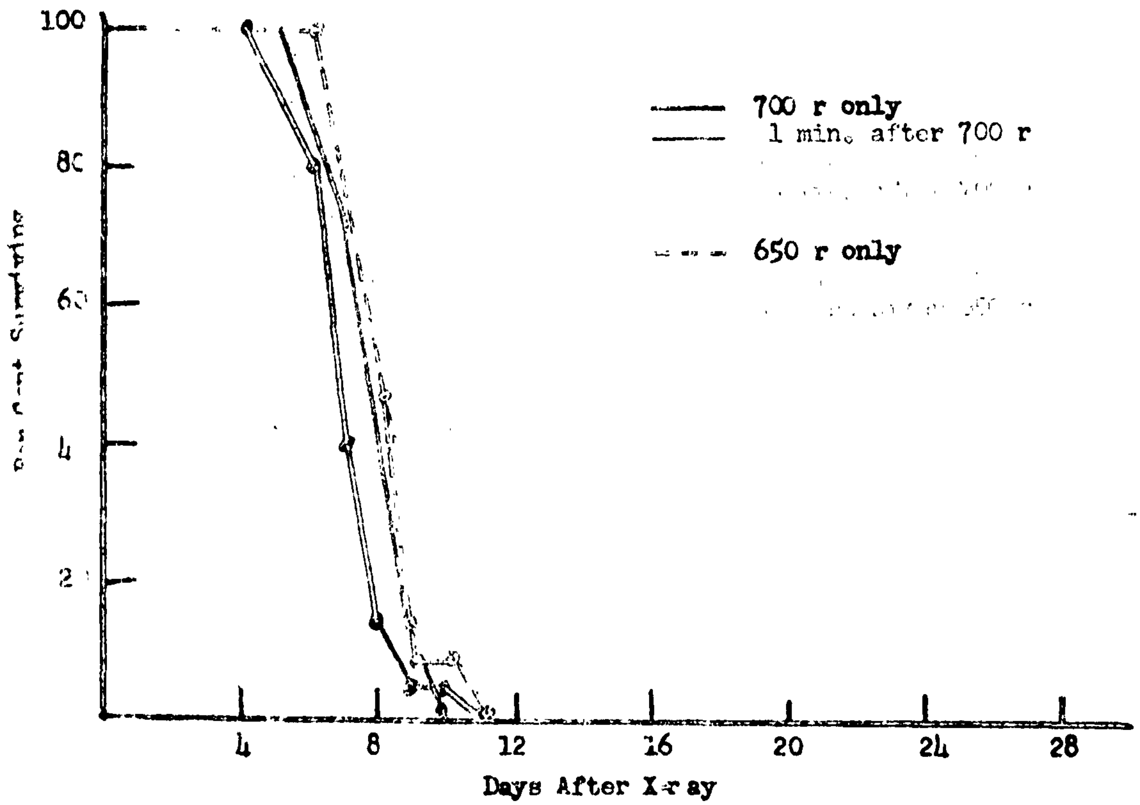


Figure 6. Effect of post-irradiation administration of 2-imino-thiazolidine-4-carboxylic acid on the survival time and mortality of CF_1 female mice exposed to 650 r or 700 r of whole body x-ray.

resulting survival curves and mortality are shown in Figure 6. From these results it is evident that the injection of thiazolidine at 30 minutes after 650 r of whole body x-ray will result in significant survival at 30 days after the irradiation exposure. Additional studies are in progress to verify and extend this unexpected observation and the results accumulated thus far are shown in Figure 7. Different numbers of animals have been used at each level of x-ray exposure in these studies. The survival curves for 550 r are based on 10 animals per group, for 600 r they are based on 17 animals, for 650 r they are based on 34 animals, and for 700 r on 31 animals in the control and treated groups.

The effects of irradiation on rat liver extracts of transsulfurase. Transsulfurase, first described by Lange (4) and named rhodanese by him, was thought to be of interest in our study because it is involved in the detoxification of cyanide in vivo.

The enzyme was extracted and concentrated according to the method of Cosby and Sumner (2) from the pooled livers of 15 adult, male and female Sprague-Dawley rats. All of the extraction procedures were carried out in the cold room at 4° C. and the final extract was stored in the refrigerator until ready for use in dilute phosphate buffer (pH 7.0). Aliquots of this enzyme preparation were removed for each study and allowed to come to room temperature before being used.

To measure the enzyme activity, aliquots of the enzyme were added to test vessels which contained a thiosulfate substrate and the potassium cyanide receptor in phosphate buffer. At measured time intervals the reaction was stopped by the addition of 38% formalin. Portions of the reactants were then added to ferric nitrate and water and the amount of thiocyanate formed was measured with a Coleman spectrophotometer Model 14 at a wave length of 490 mu against a reagent blank. All of the reactions were carried out in plastic cups formed in a lucite disc which could be positioned in the x-ray beam in a manner insuring that each cup would receive the same x-ray dose.

Initially the reaction was allowed to preceed during the radiation exposure. In subsequent studies each of the reaction components was irradiated prior to the start of the reaction. The results of these experiments are shown in Figures 8 and 9. It can be seen that the exposure of the complete reaction system to x-irradiation causes a depression of the reaction velocity and of the amount of end-product formed (Figure 8). However, when the enzyme is irradiated prior to reacting it with the complete system, the activity is increased over that of the control reaction (Figure 9). This is enhanced even further when the enzyme is irradiated in the presence of the substrate prior to reacting it with the complete system. Irradiation of the thiosulfate or cyanide, either separately or together, had no effect on the reaction velocity or on the amount of product formed. By making the reaction of the enzyme substrate-dependent, after irradiating the enzyme at various levels, and then plotting the reciprocal of the reaction velocity vs. the reciprocal of the substrate concentration, the results shown in Table 10 were obtained. From these results it appears that at x-ray levels of 1,000 r and 2,000 r there is a competitive inhibition of the enzyme. However, at a higher level of 3,000 r the mechanisms seem to be different by virtue of the fact

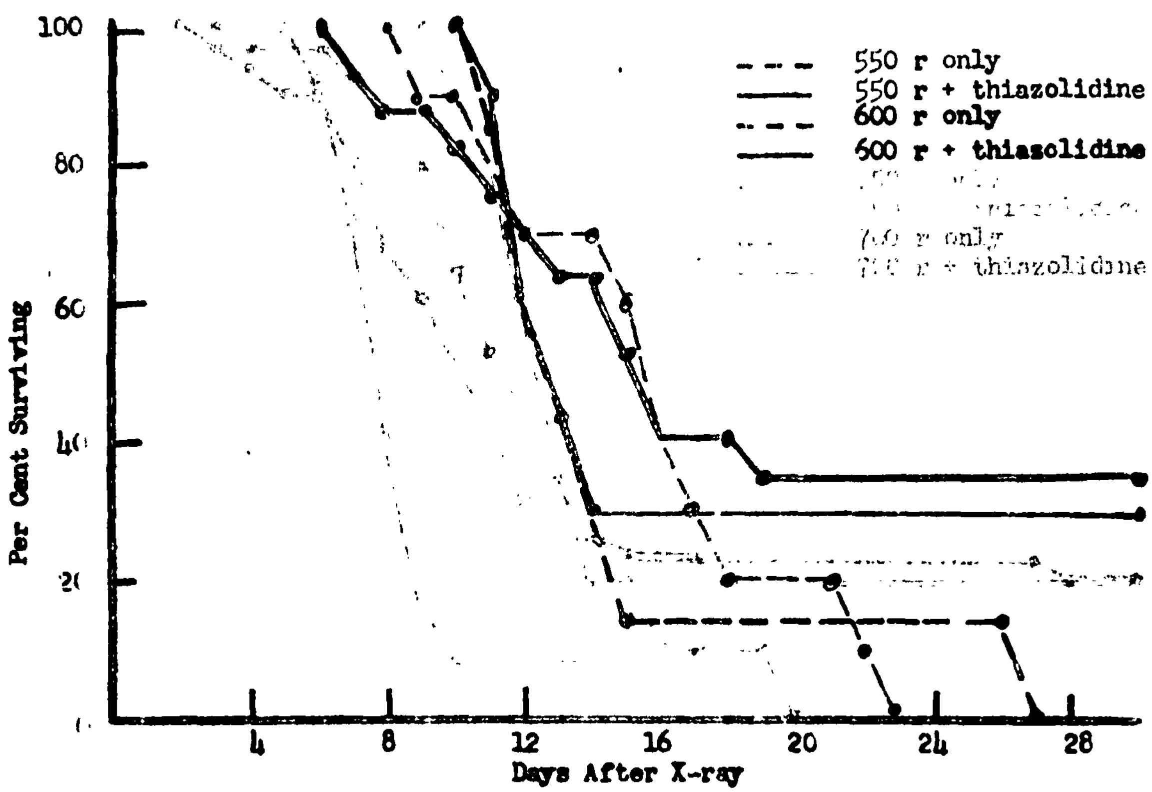


Figure 7. Effect of 2-imino-thiazolidine-4-carboxylic acid given 30 minutes after radiation exposure on the survival time and mortality of CF_1 female mice exposed to 550 r, 600 r, 650 r or 700 r of whole body irradiation.

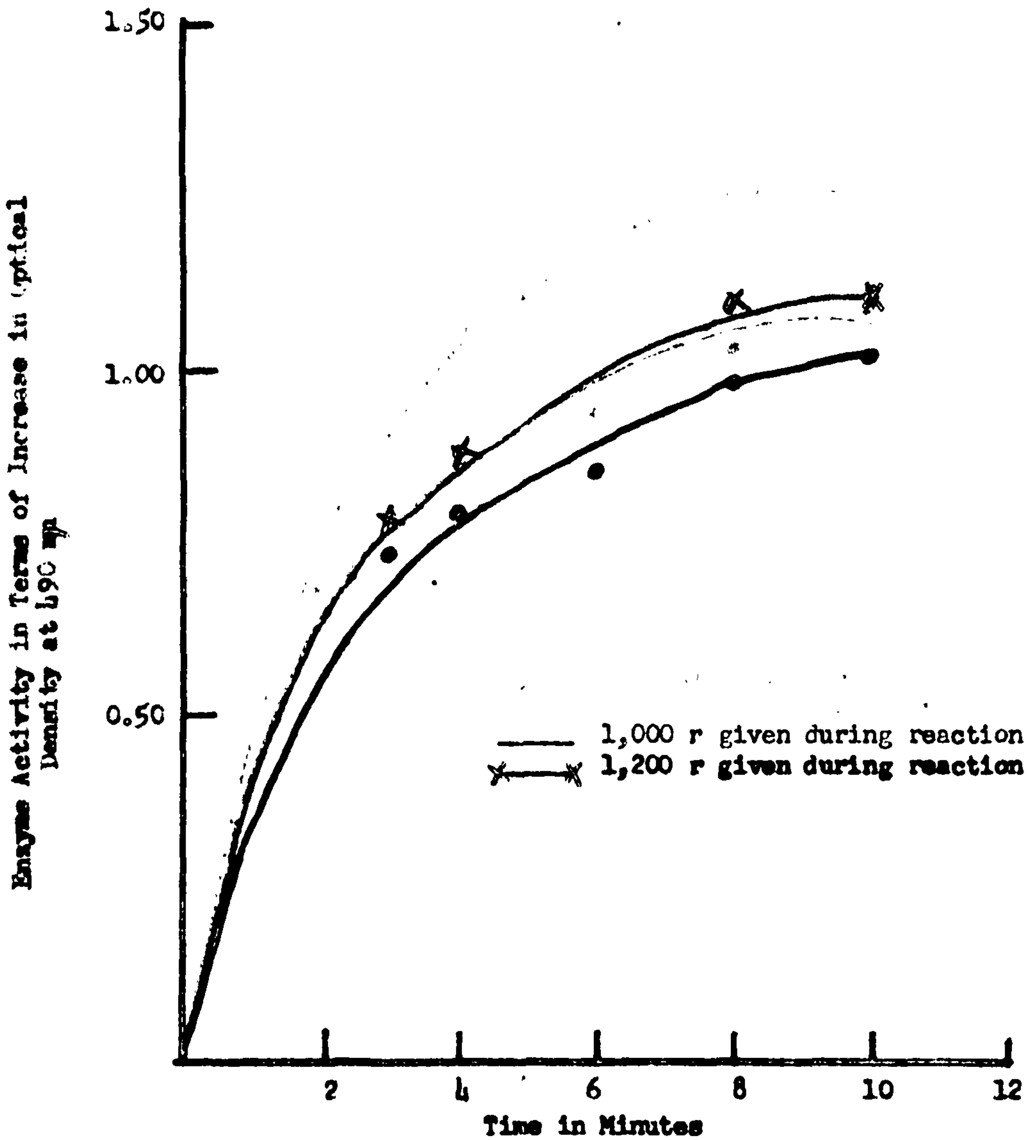


Figure 8. Effect of x-irradiation on in vitro rat liver transsulfurase activity.

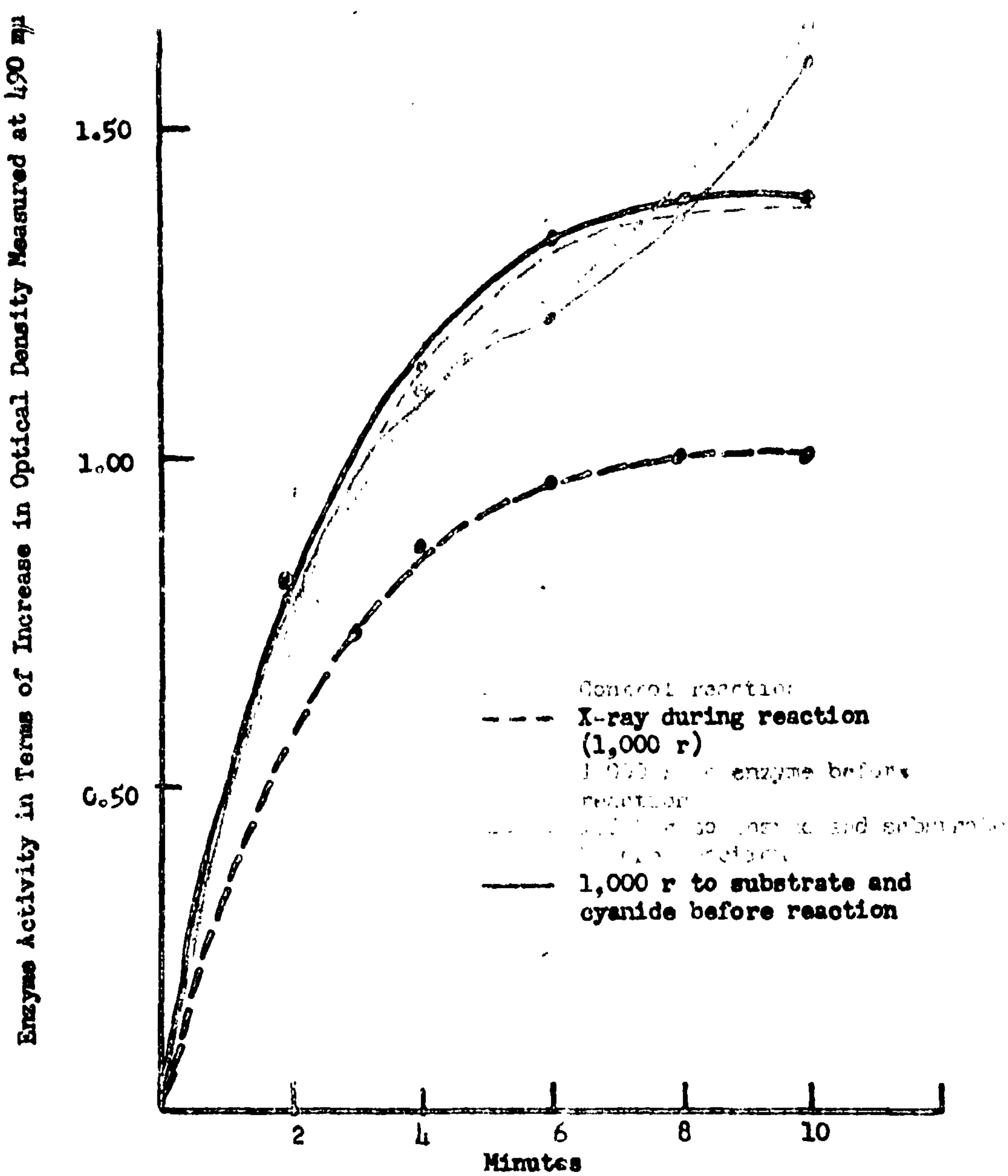


Figure 9. A comparison of the effects of radiation of the enzyme alone with the effects of irradiation of the enzyme-substrate complex on subsequent activity.

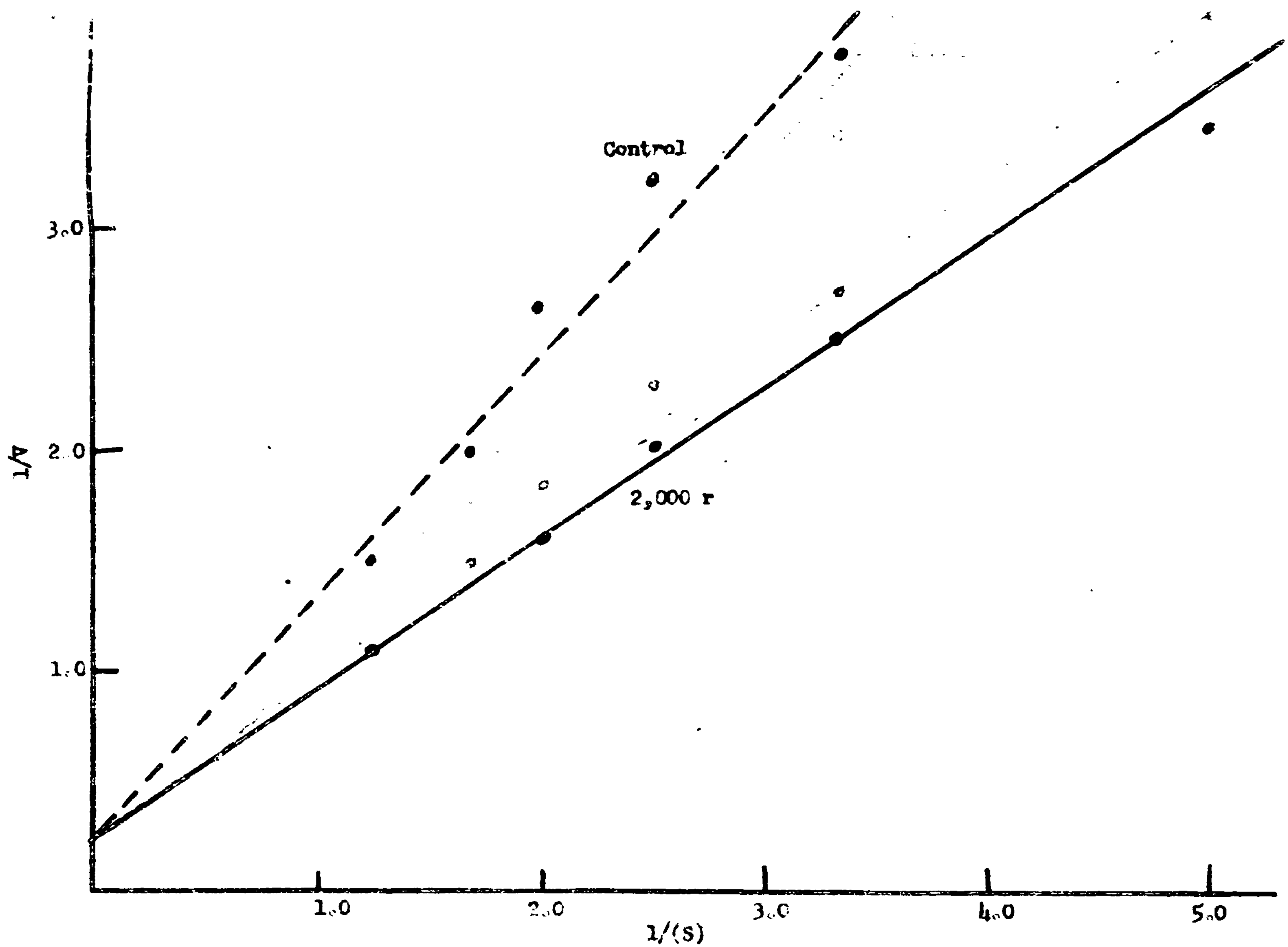


Figure 10. Michaelis-Menton plot of reaction velocity versus substrate concentration for control and x-irradiated preparations of rat liver transsulfurase.

that the intercept at the ordinate is no longer the same as that for the control reaction. Further studies are in progress to clarify these findings.

Discussion

In a previous report (1) it was shown that cyanide is a good radioprotective agent if given immediately prior to 700 r of whole body x-irradiation. However, the protective effect is no longer apparent after about ten minutes although some residual effect is obtained when the cyanide is injected 30 minutes before the radiation exposure as indicated by an increase in the median survival time of the treated mice. In the present studies an attempt has been made to show that the protective effect increases with increasing doses of cyanide but unfortunately the dose of radiation given was not high enough to clearly demonstrate this dose-effect relationship and additional studies will be required.

It has been suggested by some authors that the protection shown by cyanide is due to the anoxia which is produced at the cellular level. If this were the mechanism of protection, then it should not be possible to demonstrate a greater dose reduction factor with cyanide than is obtained by anoxia alone. Experiments are now in progress in this laboratory to compare the DRF values for cyanide and anoxia. Recently, Van der Meer (5) has suggested that the mechanism of cyanide protection is due to anoxia immediately after the radiation period and that the presence of cyanide during irradiation actually slightly sensitizes cultured cells to radiation. These conclusions are of particular interest in view of the findings in the present studies, and it is planned to carry out an investigation of the effects of anoxia after radiation exposure.

Comparative studies have been made with two cyanohydrins in an effort to find some compound within this general class which exhibits both a good protective effect and a longer duration of action. It would seem that these properties are dependent upon the ease with which nitriles release their cyanide radical, and it is of interest that pentanone cyanohydrin appeared to require more time between injection and maximum protective effect than either cyanide or acetone cyanohydrin. The dose of pentanone cyanohydrin used in this study was considerably below its toxic dose, and it is planned to repeat the study at higher dose levels.

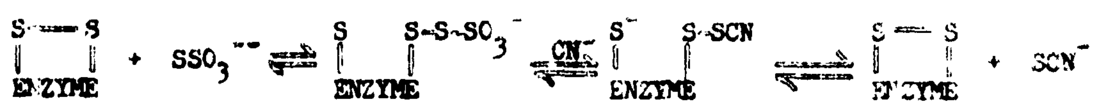
That the release of cyanide is essential for radioprotective activity is suggested by comparing the protective compounds tested in this laboratory for protection (6) with their toxicity. Williams (7) has suggested, with strong supporting evidence, that the mechanism of toxicity of the nitriles and cyanohydrins is due to one of the following type reactions:

1. $R\cdot CH_2\cdot CN \xrightarrow{NOH} CN^- + R\text{-}CH_2\cdot OH \longrightarrow SCN^- + R\cdot CHO \longrightarrow R\cdot COOH$
2. " $\xrightarrow{O}$ $R\cdot CHOH\cdot CN \longrightarrow CN^- + R\cdot CHO \longrightarrow SCN^- + R\cdot COOH$
3. " $\xrightarrow{HOH}$ $R\cdot CH_2\cdot CONH_2 \longrightarrow R\cdot CH_2\cdot COOH + NH_3$
4. " $\xrightarrow{4H}$ $R\cdot CH_2\cdot CH_2\cdot NH_2 \longrightarrow R\cdot CH_2\cdot COOH + NH_3$

Of these reactions the first two are of primary interest here because they have cyanide as an end-product of the reaction. Also there is abundant evidence that these two reactions take place in vivo. Type reaction No. 4 might possibly be of some interest in connection with radioprotection by virtue of the fact that it involves monoamine oxidase and this enzyme could play some role in the protection shown with some of the neuro-humoral agents. However, this reaction is only postulated and has not been demonstrated in vivo.

In a previous report (1) a correlation was described between the radioresistance of certain species of mammals and the activity of the transsulfurase enzyme within each member of the species. Since this enzyme is involved in the detoxification of cyanide, it was considered of interest to investigate the activity of this enzyme in relation to its response to irradiation. Since most in vitro preparations are rather resistant to radiation, the demonstration in the present study that this enzyme is fairly radiosensitive is of considerable interest.

Himwich and Saunders (8) have studied this enzyme in some detail and found that it is widely distributed in the tissues. They have estimated that in one dog liver there is enough enzyme to detoxify 4,015 grams of cyanide in 15 minutes, and enough in the skeletal muscle to detoxify another 1,763 grams. In the process of detoxification of cyanide this enzyme needs an adequate source of sulfur and this is probably the rate-limiting factor in vivo. This mobilization of sulfur substrate may play an important role in the protective mechanism shown with cyanide. Sorbö (9) has provided evidence for the disulfide bridge as the active site of the enzyme and recently Green and Westley (10) have described a double displacement reaction involving cyanide and this enzyme as follows:



It seems likely that the depressed activity observed when the enzyme reaction was undergoing irradiation in the present studies was due to the alteration of the disulfide bridge. Irradiation of the enzyme prior to reacting it with cyanide may in some way result in a lowering of the activation energy of this disulfide bridge.

The demonstration of an apparent competitive inhibition between cyanide and radiation in the present studies with the transsulfurase system is of particular interest because of the ability of the cyanide-cystine reaction mixture to reduce radiation lethality in mice when given after the x-ray exposure. Although it is tempting to speculate that these findings are related and that the post-irradiation protective effect of the thiazolidine derivative is due to the reversal of a radiation-inhibited system, this explanation would not be consistent with the finding that the protective effect is not present when the agent is given immediately after the x-ray exposure rather than at 30 minutes after radiation. Additional studies are needed in order to determine whether the 30-minute delay between radiation and the administration of the thiazolidine derivative is highly critical and these studies are now in progress. If the delay period is sharply restrictive then the possibility that the protective effect is due to a reduced synthesis of some toxic substance or the blocking of the production of some injurious agent (the so-called lethal synthesis) must be considered.

The finding that the post-irradiation administration of the cyanide-cystine reaction mixture decreases radiation lethality in mice is also of particular interest in explaining some of our previous findings (1). Although the maximal radiation protective effect of cyanide was obtained in our previous studies when the agent was given immediately prior to the radiation exposure, there was an indication (increased median survival time) of a radioprotective effect when the cyanide was given at 30 minutes prior to the radiation dose. This effect was also fairly critically related to the time of administration, since it was not detected when the cyanide was given at 20 minutes or at 40 minutes prior to the x-ray exposure. If the mechanism of this effect of cyanide is related to the post-irradiation protective effect of the cyanide-cystine reaction mixture, then it would seem to be unlikely that this mechanism involves a stimulation of the existing bone marrow stem cells to produce an earlier repopulation of the hematopoietic system in the irradiated animal. Compounds which contain sulfhydryl groups often have a unique ability to promote wound healing, and it is possible that the cyanide acts in some manner to stimulate the healing and repair mechanisms in the irradiated animal and that the thiazolidine derivative exerts a similar function when given after the radiation exposure. However, this mechanism also fails to provide a reasonable explanation for the apparent dependence of the protective effect on the time of administration of the agents. If cyanide acts by causing anoxia during the immediate post-irradiation period as suggested by Van der Meer et al. (5), it is difficult to understand how it could exert a protective effect when it is given 30 minutes prior to the radiation exposure and not when given at either 20 or 40 minutes before the radiation. Since high pressure oxygen during the x-ray exposure reduces the radioprotective effect of cyanide given immediately prior to the x-ray exposure (1), it is of interest to determine whether this treatment also reduces the protective effect of cyanide given 30 minutes prior to the x-ray exposure and these studies and other studies designed to clarify the mechanism of the protective effect of both cyanide and the thiazolidine derivative have been initiated.

Summary

1. The pre-irradiation administration of potassium cyanide at dosage levels equivalent to 2.25 mgm./kgm. of HCN increase the LD_{50} of acute whole body x-ray exposure from about 500 r to over 900 r in CF_1 female mice.

2. The ability of various cyanide producing nitriles to protect mice against lethal doses of whole body x-irradiation appears to depend on the ease with which they release free cyanide.

3. The *in vitro* activity of rat liver transsulfurase is inhibited when the enzyme is exposed to x-ray doses of 800 r, 1,000 r or 1,200 r in the presence of a substrate and cyanide, whereas radiation of the enzyme

alone or in combination with the substrate only results in enhancement of its activity.

4. A minor metabolite of cyanide, 2 imino-thiazolidine-4-carboxylic acid, has been prepared by reacting cyanide and cystine and found to be capable of reducing radiation lethality in CF_1 female mice when given at 30 minutes following whole body x-ray exposures in the range of 550 r through 700 r.

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PHARMACOLOGICAL AND TOXICOLOGICAL COMPOUNDS AS PROTECTIVE OR THERAPEUTIC AGENTS AGAINST RADIATION INJURY IN EXPERIMENTAL ANIMALS

III. Radioprotective Effects in Proton-Irradiated Mice Pretreated with Chemical Protectors

D. G. Oldfield, J. Doull, V. Plzak, A. Hasegawa and A. Sandberg

This report concerns: Radioprotective effects in mice total-body irradiated by high energy (440 Mev) protons following pre-irradiation treatment with 2-mercaptoethylamine hydrochloride (MEA) and with p-aminopropiophenone (PAPP), and a comparison with similar effects in mice irradiated by 250 Kv x-rays.

Immediate or ultimate application of the results: This investigation has relevance to at least three significant radioprotection problems. These are, first, the extent to which a selected agent can modify the response of a biological system to one particular type of radiation--specifically, high energy protons; second, the relative effectiveness of two different types of radioprotective agents in modifying the response of a biological system to this type of radiation; and third, the relative effectiveness of one type of radioprotective agent compared in systems exposed to two different types of radiations--protons and x-rays. Interrelated problems of this type are usually best attacked using a comprehensive experiment specifically designed for the purpose. This report presents solutions of some of the methodological and technical problems arising in the design and execution of such an experiment which should be useful for future investigations in this laboratory or elsewhere. Specifically, methods of proton and x-ray dosimetry using a Victoreen cavity ionization chamber are presented. Also, certain aspects of the problem of analyzing radioprotection data in populations of treated animals are discussed. Specifically, the analysis indicates that pre-irradiation treatments with MEA and with PAPP are able to reduce 30-day lethality in mice exposed to high energy protons.

* * * * * * * *

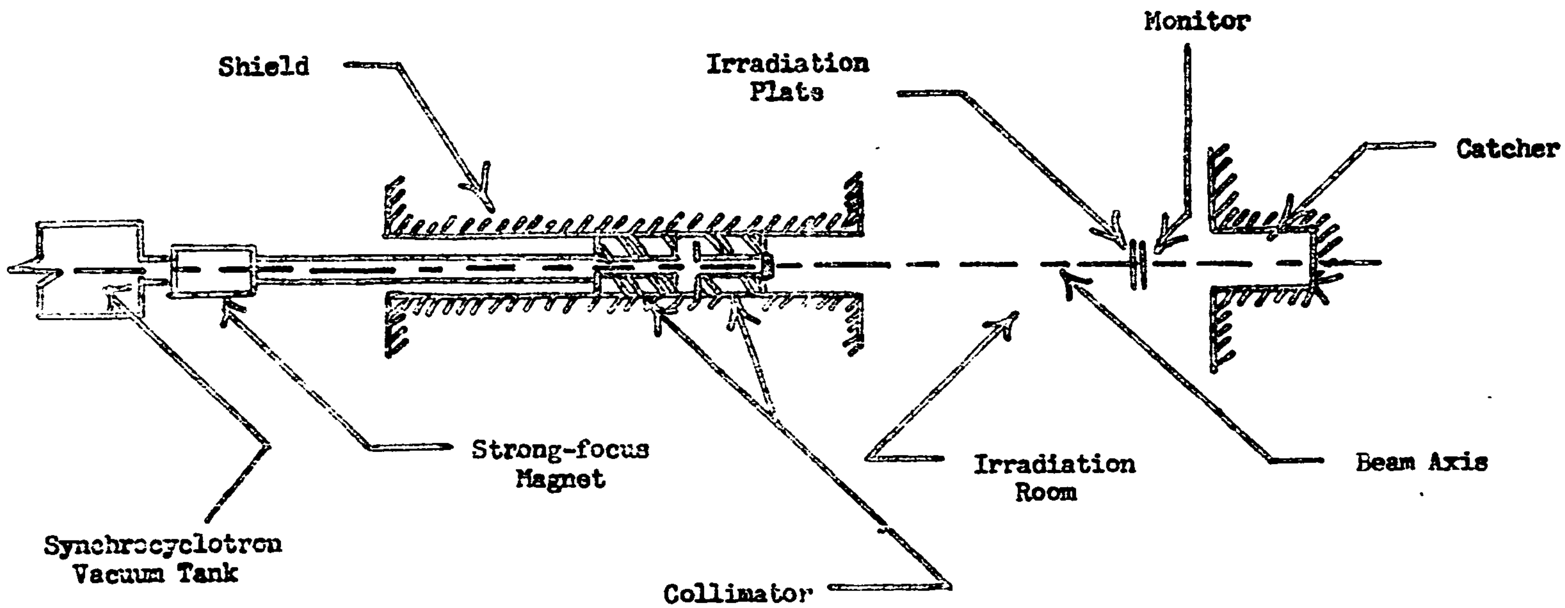
Relatively little published work in any phase of proton radiobiology exists. Tobias, Anger and Lawrence (1) measured the dose of 315 Mev protons producing 50% lethality at 30 days in whole-body irradiated strain A mice. They obtained a value of 620 rads and an RBE of approximately 1.1 compared with 180 Kv x-rays. Warenaw and Oldfield (2) determined the effectiveness of 90 Mev protons relative to 250 Kv x-rays in producing weight loss in the spleen and thymus of whole-body irradiated LAF_1 mice. Assuming an exponential regression of 5-day residual organ weight on dose, protons were found to be more effective than x-rays by a factor of about 2. Kurlyandskya and coworkers (3) recently reported an $LD_{50/30}$ for white male mice irradiated with 660 Mev protons of about 1050 rads. The RBE relative to 180 Kv x-rays was reported to be about 0.55. These workers also reported that various mercapto compounds increased the number of survivors.

Some studies of localized proton irradiation of mammals have been made, the intent being to selectively destroy cells only in certain limited regions of the body (4-11). Larsson and Kihlman (12) investigated the production of chromosome aberrations in the plants Allium and Vicia, finding a relative effectiveness of 0.7 for 170 Mev protons relative to 180 Kv x-rays. Oldfield (13) investigated the effectiveness of low-energy protons relative to cobalt-60 gamma rays for the production of tumoricidal effects on an Ehrlich ascites carcinoma irradiated in vitro and then grown intraperitoneally in CF_1 mice. This sensitive method (14) yielded an RBE of unity. Although a detailed literature search has not been made, it would appear that the number of published works in the field of radioprotection against high-energy proton radiation is rather small.

Physical materials and methods: Proton beam geometry and properties. The source of high energy protons used in this experiment was the University of Chicago synchrocyclotron. As shown in Figure 1, the magnetically extracted proton beam emerges from the vacuum tank of the synchrocyclotron, passes through a strong-focus magnet and aluminum vacuum pipe, and enters the irradiation room. In the irradiation room, the beam passes through air until striking the irradiation apparatus and monitoring ionization chamber. Beyond the chamber, the beam is absorbed in concrete at the base of a blind collimator ("catcher").

The proton beam produced by the synchrocyclotron is pulsed, having a duration of 400 microseconds per pulse and a repetition rate of 70 pulses per second. Extraction of the beam commences when an energy of 440 ± 5 Mev is reached. The trajectory of protons having this energy was checked by floating wire measurements carried out just prior to the present series of runs. The strong-focus magnet consists of one 24-inch center section and two 12-inch end sections. Separation between sections is 12 inches. Each section is a hyperbolic quadrupole lens; the vertex-to-vertex distance of poles lying on opposite sides of the lens axis is 4 inches. The main focusing current to the strong-focus magnet is supplied by a servo-regulated power supply. The three sections of the magnet are series connected with the supply. In addition, variable external resistors shunt each section, allowing the focusing strength of the center section to be changed relative to the end sections. The current in the center section, and that in the end sections, was monitored potentiometrically from the voltage drops produced across a calibrated manganin resistor before and during each biological run.

The irradiation room is separated from the synchrocyclotron by a concrete, earth, and steel shield into which the vacuum pipe extends. Two pairs of limiting apertures exist along the length of the vacuum pipe. These consist of the entrance and exit pole tips of magnets not used in the present experiment. Taken together, these apertures constitute a tapered collimator, 150 inches long and roughly 3 inches by 3 inches at the input end and 4 inches by 4 inches at the output end.



Elevation in plane of beam axis showing geometry for proton irradiation. 1/16" = 1' along axis. Unscaled perpendicular to axis.

Figure 1. Diagram of exposure and monitoring positions.

In passing from the vacuum tank to the irradiation apparatus, the proton beam passes through 0.06 gm./cm.^{2} mylar, comprising vacuum pipe windows and through 1.34 gm./cm.^{2} of air, most of the latter in the irradiation room. For protons of 440 Mev, these extraneous absorbers produce almost negligible energy loss, but statistical fluctuations in energy loss will result in some additional spread of proton energies initially present in the beam. Scattering from collimator edges, vacuum piping, etc. will also introduce low energy protons into the beam. However, the total energy spread present at the irradiation apparatus is not expected to exceed 5%. In addition to energy dispersion, interactions of protons with mylar, air, and other materials in the beam path results in neutron production by charge-exchange and other nuclear reactions. Since neutron contamination of the proton beam would be expected to produce a rather diffuse and poorly defined beam, the relatively sharp fall-off at the edge of the beam seen using photographic film indicates a low degree of contamination.

The irradiation apparatus consisted of a 8-1/2 x 8-1/4 x 1/4 inch thick lucite plate attached to an aluminum frame which during irradiation moved up and down in a plane perpendicular to the beam axis. The frame is driven through a crank-and-link mechanism by a motor, the frame making one complete oscillation each six seconds. The excursion of the frame during oscillation is 2-1/2 inch each side of the mid-position. Mice to be irradiated were placed in vented celluloid centrifuge tubes (1-1/8" O.D. x 3-7/8" long) and each of the latter secured to the up-beam surface of the lucite plate by a pair of thin rubber bands.

Focusing the proton beam on the irradiation apparatus using the strong-focus magnet gave a beam roughly rectangular in shape (vertical height of 4 inches and a lateral width of 9 inches). By having the lucite plate move up and down across the axis of the fixed beam, the dose rate was reduced to a more suitable value, the vertical uniformity of the beam over the surface of the lucite plate was improved, and the field size was increased by about 5 inches in the vertical direction. Initial estimates of beam size, shape, uniformity, etc. were made using photographic film (DuPont, Type 1290).

Downbeam of the irradiation apparatus an argon-filled (4 pounds per square inch, gauge) transmission ionization chamber with aluminum windows and electrodes (4-1/2 inch diameter) monitored a portion of the beam passing through the lucite plate. The chamber was polarized to 150 v/cm. and collected electrons. Coupled to the chamber was a vibrating reed electrometer (Applied Physics Corporation) with capacitor input; the output of the electrometer was fed to an extended-range recorder. Thus, the charge produced in the chamber during irradiation was collected continuously and cumulatively during each irradiation run. The voltage corresponding to the total charge collected at any time was displayed on the recorder, permitting, after calibration of the monitoring apparatus, the accurate delivery of any desired dose. The calibration of the beam monitoring apparatus is described in the next section.

The "catcher" absorbs the beam after it has passed through the irradiating and monitoring apparatus. The catcher also reduces the diffusion of thermal neutrons (produced when the proton beam is absorbed) back towards the irradiation and monitoring apparatus.

Proton dosimetry and monitor calibration. Measurement of absorbed proton dose was based on (1) the use of a Victoreen cavity-ionization chamber and associated charger as a charge-measuring rather than a roentgen-measuring device, and (2) the use of the known exposure dose rate produced by a Co-60 source to calibrate this charge-measuring device. The quantitative basis of the measurements is as follows:

Let

$\dot{R}_\gamma$ roents/min = known equilibrium exposure dose rate in air produced by the gamma source.

V cm^3 = geometric volume of the chamber cavity.

ρ_γ gm/cm^3 = density of air in the volume V when the gamma measurement is made.

t_γ min = exposure time for gamma irradiation of the cavity.

From the definition of the roentgen, we have the factor

$$K_R = \frac{1}{.001293} \text{ statcoulombs/gm. air/roentgen}$$

Then if the Victoreen is placed in the field $\dot{R}_\gamma$ of the gamma source for a time t_γ, the total charge Q_γ produced under conditions of electronic equilibrium is

$$Q_\gamma \text{ coulombs} = K_q K_R \rho_\gamma V \dot{R}_\gamma t_\gamma$$

where $K_q = 1/(2.9980 \times 10^9)$ coulombs per statcoulomb. If the Victoreen charger measures charge in scale units S, and if the charger response is linear, then $S = kQ$, where k is a constant of proportionality. Therefore, from the gamma irradiation we find

$$k = \frac{S_\gamma}{K_q K_R \rho_\gamma V \dot{R}_\gamma t_\gamma}$$

the only unknown quantity being the cavity volume V.

Consider now a tube of unit density material, assumed to be water, placed in a uniform beam of high energy protons. Assume that the axis of the tube is perpendicular to the beam axis, and that the diameter of the tube is much smaller than the range of the protons. The dose produced in the water will be predominantly that due to energy loss by ionization and, since proton scattering at these energies is not large, will be approximately uniform over the tube. If a small volume of water in the tube is replaced by the same volume of air, the dose produced in the water, D_p, is related to the dose now produced in air, D_p', by $D_p = sD_p'$, where s is the relative stopping power of water to air for protons of specified energy. If the air-filled cavity of the same Victoreen chamber used above is introduced into the tube of water and irradiated until the monitoring apparatus has accumulated M units of voltage, the total charge Q_p coulombs produced in the air in the cavity is related to the absorbed dose by

$$D_p \text{ rads} = sQ_p \frac{w}{e} \frac{K_\varepsilon}{K_r} \frac{1}{\rho_p V} \quad \text{(for M units of monitor voltage)}$$

where

ρ_p gm./cm^3 = density of air in volume V during proton irradiation.

w = electron volts (ev) expended per ion pair (ip) produced = 33.3 ev/ip for 340 Mev protons in air (15).

e = 1.6020×10^{-19} coulomb/ip.

K_ε = 1.6020×10^{-12} erg/ev.

K_r = 100 ergs/gram/rad.

The basic assumption made is that the introduction of a short length of the Victoreen into the proton radiation field alters the spectrum of incident protons only negligibly, and that the metal shell of the Victoreen does not contribute appreciably to the spectrum of secondary electrons. For high energy protons these assumptions are plausible.

If the charge Q_p produces in the charger a deflection of S_p scale units, then $Q_p = S_p/k$. Substituting this expression together with the value of $\underline{k}$ found previously into the expression for D_p, the cavity volume V cancels, and we obtain the calibration factor.

$$F \frac{\text{rads}}{\text{monitor unit}} = \frac{D_p}{M} = s \frac{S_p}{M} \frac{w}{e} \frac{K_\varepsilon K_q K_R}{K_r} \frac{\mathring{R}_\gamma t_\gamma}{S_\gamma} \frac{\rho_\gamma}{\rho_p}$$

The value of the relative stopping power $\underline{s}$ was obtained from the individual stopping powers given in range-energy tables (16), and found to be 1.138. Air density was calculated on the assumption of dry air in the Victoreen chamber cavity. In this case the ratio of air densities ρ_γ/ρ_p can be replaced by $P_\gamma T_p/P_p T_\gamma$ where P is the pressure and T is the absolute temperature. Inserting numerical values, we find.

$$F = 0.846 \frac{S_p}{M} \frac{T_p}{P_p} \cdot \frac{\mathring{R}_\gamma t_\gamma}{S_\gamma} \frac{P_\gamma}{T_\gamma}$$

The gamma source used in the calibration was the Argonne Cancer Research Hospital Revolving Cobalt-60 Therapy Unit (17). The exposure dose rate $\mathring{R}_\gamma$ at the center of rotation at time of calibration (November 31, 1962) was

46.6 r/minute. A 250 r Victoreen chamber with equilibrium lucite cap was used with a Model 70 Victoreen charger in a 10 cm. x 10 cm. radiation field.

The Victoreen chamber and charger calibrated with the gamma source were used to measure doses at the center of each of ten of the twelve tube positions on the face of the lucite irradiation plate, as shown in Figure 2. Measurements were not made at positions 9 and 10 due to the likelihood of stem leakage in the Victoreen at these positions. For the upper positions, measurements were made with the Victoreen chamber tip at the center of both air-filled and water-filled tubes. Prior to immersion in water, the tip of the chamber was covered by a thin, rubber finger cot, stretched tightly and taped to the metal shell of the chamber to prevent water leakage into the chamber. For the lower positions, only measurements with air in the tubes were feasible; these values were corrected to water values using the water/air ratios obtained from measurements in the upper positions. The values of F obtained are also shown in Figure 2. Since the variation in dose was found to be least between upper and lower levels at corresponding lateral positions, and since the variation decreased toward the center of the beam, position 9 was assumed to have the same F value as position 3; and position 10, the same value as position 4. Positions 5, 6, and 12 were regarded as having values too divergent from the others and were not used. The remaining nine positions were grouped into three sets of three positions each. Mean values of F, the per cent standard deviation of the mean σ, and the per cent standard deviation of individual F values from the mean σ_1 are shown in Table 1. It can be seen that using the mean F for the separate sets would result in a relatively large dispersion for the last set (10.4%). Therefore, the mean value of F for all positions were used.

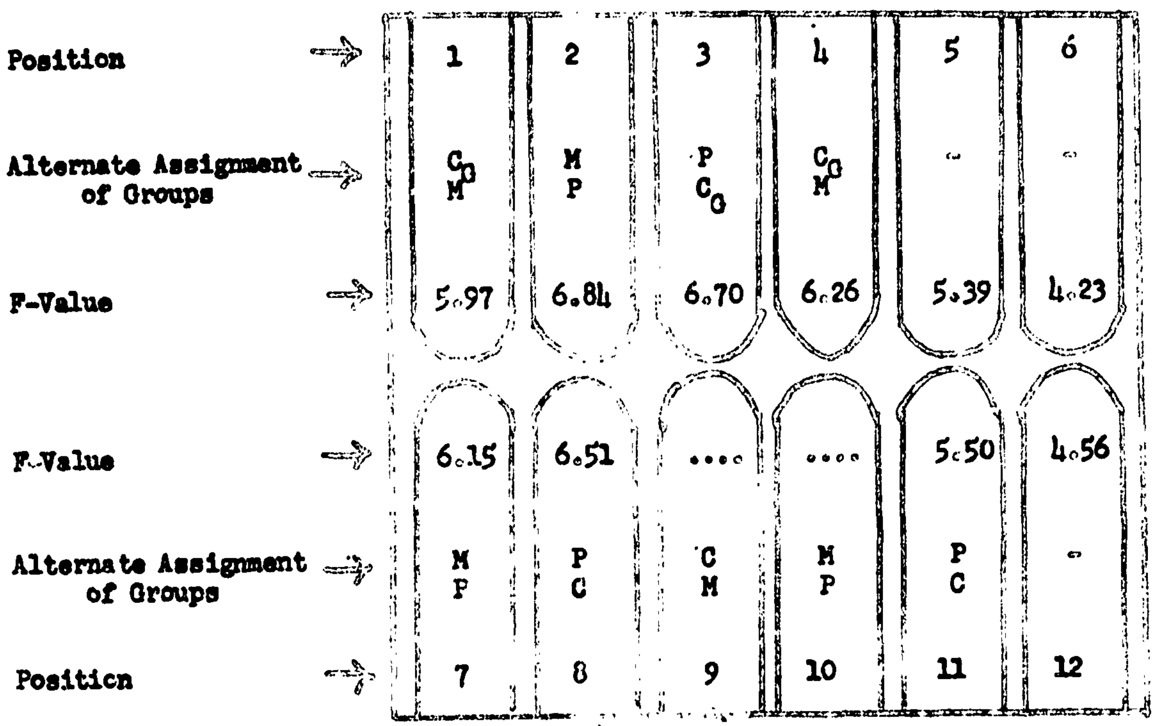
TABLE 1

OVERALL AND GROUP CALIBRATION FACTORS FOR PROTON EXPOSURE POSITIONS

Positions	F	σ	σ_1
All	6.32	2.2%	6.7%
1,4,9	6.31	3.4%	5.9%
2,7,10	6.42	3.4%	5.9%
3,8,11	6.23	6.0%	10.4%

X-ray beam geometry and dosimetry. Specifications of the x-ray beam were as follows:

Generator, General Electric Maximar Type III;
Energy, 250 Kvp;
Tube current, 15 ma;
External filter, 1/4 mm. copper plus 1 mm. aluminum;
FSD, 75 cm.;



C = Control water
C_G = Control propylene glycol
M = MEA
P = PAPP

Figure 2. Lucite irradiation plate for proton irradiation exposures showing positions of mouse tubes and dose distribution.

Backscatter material, lucite;
External collimator, none;
Beam diameter, approximately 30 cm.

The beam was directed vertically down with axis normal to the backscatter surface. A 1000-r Victoreen chamber placed 23.5 cm. from target and off-axis together with remote amplifier and meter were used to monitor the dose rate during all runs. Tube current was manually adjusted to maintain the dose rate constant to be about 3%. Tube voltage was monitored at the high-voltage transformer primary. Voltage was maintained constant to about 3%.

A 1-inch thick by 13-inch diameter lucite disc, centered on the geometric beam axis, rotated at about 1 rpm during irradiation. The disc was scalloped out along 16 equally spaced radii to accommodate 16 tubes containing mice to be irradiated. The centers of the tubes fell on a circle of 22 cm. diameter, concentric with the disc. An insulated box 2 feet square and 1 foot deep centered on the disc and having an open top maintained a constant temperature of 27° C. at the disc surface.

The methodology of the x-ray dosimetry is very similar to that for the protons. Using the same notation as before, but with the subscript "x" denoting x-rays, we have

$$\dot{R}_x \; \frac{\text{roent}}{\text{min}} = \frac{S_x}{t_x} \; \frac{T_x}{P_x} \; \frac{\dot{R}_\gamma t_\gamma}{S_\gamma} \; \frac{P_\gamma}{T_\gamma} \; .$$

S_x is measured with the same cavity chamber and charger used previously. The chamber tip is fixed at the center of a tube fitted with unit density material, assumed to be water. The tube plus chamber are placed in one of the 16 positions on the lucite disc and rotated during irradiation. The average absorbed dose rate is then given by $\dot{D}_x$ (rad/min) = $\bar{f}\,\dot{R}_x$ where $\bar{f}$ is a roentgen-to-rad conversion factor averaged over the energy spectrum of x-rays present at the point of interest. An approximate value of $\bar{f}$ can be found when the HVL of the x-ray beam is known.

Measurement of the HVL of the x-ray beam specified above gave a value of 1.01 mm. of copper. Using this value, $\bar{f}$ for water is found from calculations reported in the literature (18) to be 0.952 rads/roentgen. Inserting this value and the cobalt-60 data in the equation, we find

$$\dot{D}_x \; \frac{\text{rads}}{\text{min}} = 2.706 \; \frac{S_x}{t_x} \; \frac{T_x}{P_x}$$

In the actual measurement, the tube was packed with a section of water-filled dialysis tubing with ends tied off, and the cavity Victoreen chamber tip (sealed with a finger cot as previously) inserted into an invaginated section of the tubing. The reproducibility of the measurements made in this way (0.5%) was considerably better than the constancy of the x-ray tube current (3%).

<u>Biological materials and methods</u>. Mice used in this experiment were Carworth Farms CF_1, caged not more than 12 per stainless steel cage (7" H, 9" W, 13" L) in which food (Rockland Mouse Diet) and drinking water were available <u>ad libitum</u>. All cages were housed in a single air-conditioned room with temperature held at 25 ± 2° C. The cages were inspected for dead mice daily, and cleaned weekly. Both proton and x-ray irradiations were carried on during three successive 6 to 8 hour periods spaced 24 hours apart. For the first period, male mice 19 ± 1 weeks of age were used; for the second period, male mice 17 ± 1 weeks of age were used; for the third period, male mice 15 ± 1 and female mice 15 ± 1 weeks of age were used. The mice used in any particular period were randomized just prior to the beginning of that period, male and female groups used in the third period being randomized separately and the groups kept separate.

Chemical agents used in this study were made up in concentrations that permitted the use of an injection volume corresponding to less than 2% of the body weight of the mouse. PAPP was prepared by dissolving C.P. grade reagent in propylene glycol with gentle heating and then diluting with an equal volume of triple-distilled water. The concentration of the final solution was 3 mgm./milliliter. A fresh solution was prepared each 2-3 hours. MEA was prepared by dissolving the C.P. grade reagent in triple-distilled water. The concentration of the final solution was 22.5 mgm./ml. A fresh solution was prepared each hour. Control mice received one or the other of the above diluents, as described below.

In the proton irradiations, mice were processed in batches of nine: three mice received 30 mgm./kgm. of PAPP, three mice received 22.5 mgm./kgm. of MEA, two mice received water only, and one mouse received the propylene glycol-water mixture only. The following batch of nine was prepared identically, except that two mice received propylene glycol-water mixture only and one mouse received only water. All succeeding batches alternated in this way.

Injections were given intraperitoneally using a 1 ml. syringe and 26 gauge needle, 1/2 inch long. After injection, mice were placed in vented tubes. A delay of five minutes was interposed between the end of the injections and the start of irradiation, during which time the tubes were placed at specific locations on the lucite plate. The placement of tubes alternated between the two configurations shown in Figure 2 to obtain good dose uniformity. As mentioned previously, the plate containing tubes and mice moved vertically up and down past the fixed beam continuously during irradiation. After delivering a measured radiation dose to this batch of mice, the tubes were removed, and the mice sorted into appropriate cages. The propylene glycol and the water control mice were caged together, but were permanently marked so as to be distinguishable. The female control mice were given water only.

The x-ray irradiations commenced one week after the end of the proton irradiations. The biological procedures used for irradiations were exactly comparable with those used for proton irradiations, except that the mice were processed in batches of twelve.

Results

The cumulative per cent mortality after 30 days, p, is shown in Table 2. The standard deviation is calculated on the assumption that deaths within each group of mice at a particular dose level obey binomial statistics, and that the per cent mortality observed in this sample is a reasonable estimate of the per cent mortality that would be observed in a large population of mice. The number n is the original number of mice irradiated, not including any that died during irradiation or during the first two days post-irradiation. In the control groups, the number of mice excluded from analysis due to immediate death (during irradiation) or due to short-term death (two days post-irradiation) is negligible. In groups receiving MEA before irradiation, the excluded mice comprise about 5-10% of the initial number; in groups receiving PAPP, about 10-15%. The three age groups irradiated during the three successive treatment periods all exhibited essentially the same survival behavior and the groups were pooled for analysis.

The data in Table 2 is plotted in Figures 3 through 10, inclusive, in probit units of mortality versus dose. The straight-line fits to these data have been estimated by eye. When additional data at higher doses are obtained from experiments now in preparation, a maximum-likelihood fit of the data will be calculated. It should be possible from such calculations to determine in an unambiguous way whether the linear regression of mortality is on dose, logarithm of dose, or some other function of dose. A plot of the present data against log dose did not reveal any striking improvement in linearity.

For each estimated straight-line fit to the data, the dose producing 50% mortality, D, and the slope of the line, b, measured in probits per rad, can be obtained. These values are collected in Table 3.

The dose reduction factor, DRF, is a convential and useful index of protector effectiveness independently of any further implications that the mortality curves providing that number may have. The same may be said for the relative biological effectiveness, RBE. When protection against two different types of radiation afforded by a single protector are being compared, a logical extension of DRF and RBE indices is obtained by defining relative protective effectiveness (RPE) as the ratio of doses of two different types of radiation producing 50% mortality by 30 days when the same protective substance is used in the same way against both radiations. An RPE is essentially an RBE modified by the use of the protector. With this orientation, the values in Table 4 have been calculated from those of Table 3.

An RBE of 0.72 is not unexpected since the linear energy transfer (LET) of 440 Mev protons is about 0.29 Kv/micron while that for the x-rays used here is about 4-5 times this value. Both PAPP and MEA produce DRF's appreciably greater than unity. However, while for protons the MEA appears more effective than PAPP, for x-rays the situation is reversed. Differences in protection between the compounds can be seen also from the RPE values for which a factor 2 difference exists.

TABLE 2

THIRTY-DAY MORTALITY DATA FOR CF_1 MICE EXPOSED TO VARIOUS DOSES OF PROTON OR X-IRRADIATION

Dose (rads)	Control			PAPP			MEA			Control (Female)		
	n	p%	$\pm\sigma$	n	p%	$\pm\sigma$	n	p%	$\pm\sigma$	n	p%	$\pm\sigma$
440 Mev Protons												
235	35	0	1.4	36	0	1.4	41	2.4	2.4	..	....	....
351	36	13.9	5.8	37	2.7	2.6	37	0	1.4	18	0	2.8
468	36	8.3	4.6	35	0	1.4	37	0	1.4	18	5.6	5.4
591	36	25.0	7.2	35	14.3	5.9	35	11.4	5.4	18	50.0	11.8
702	35	51.4	8.4	34	17.6	6.5	37	13.5	5.6	18	44.4	11.7
820	36	55.6	8.3	34	14.7	6.1	35	17.1	6.4	..	....	....
939	35	71.4	7.6	36	47.2	8.3	34	23.5	7.1	..	....	....
250 Kvp X-rays												
222	36	2.7	2.7	..	....	...	..	....	...	..	....	....
333	35	14.3	5.9	..	....	...	..	....	...	18	5.5	5.4
444	36	27.7	7.5	38	13.2	5.5	41	2.4	2.4	18	11.0	7.4
555	36	66.7	7.9	40	12.5	5.2	40	5.0	3.5	18	44.4	11.7
666	36	66.7	7.9	36	25.0	7.2	38	28.9	7.3	18	83.3	8.8
777	..	....	...	35	31.4	7.8	41	39.0	7.6	..	....	....
888	..	....	...	36	27.8	7.5	41	51.2	7.8	..	....	....

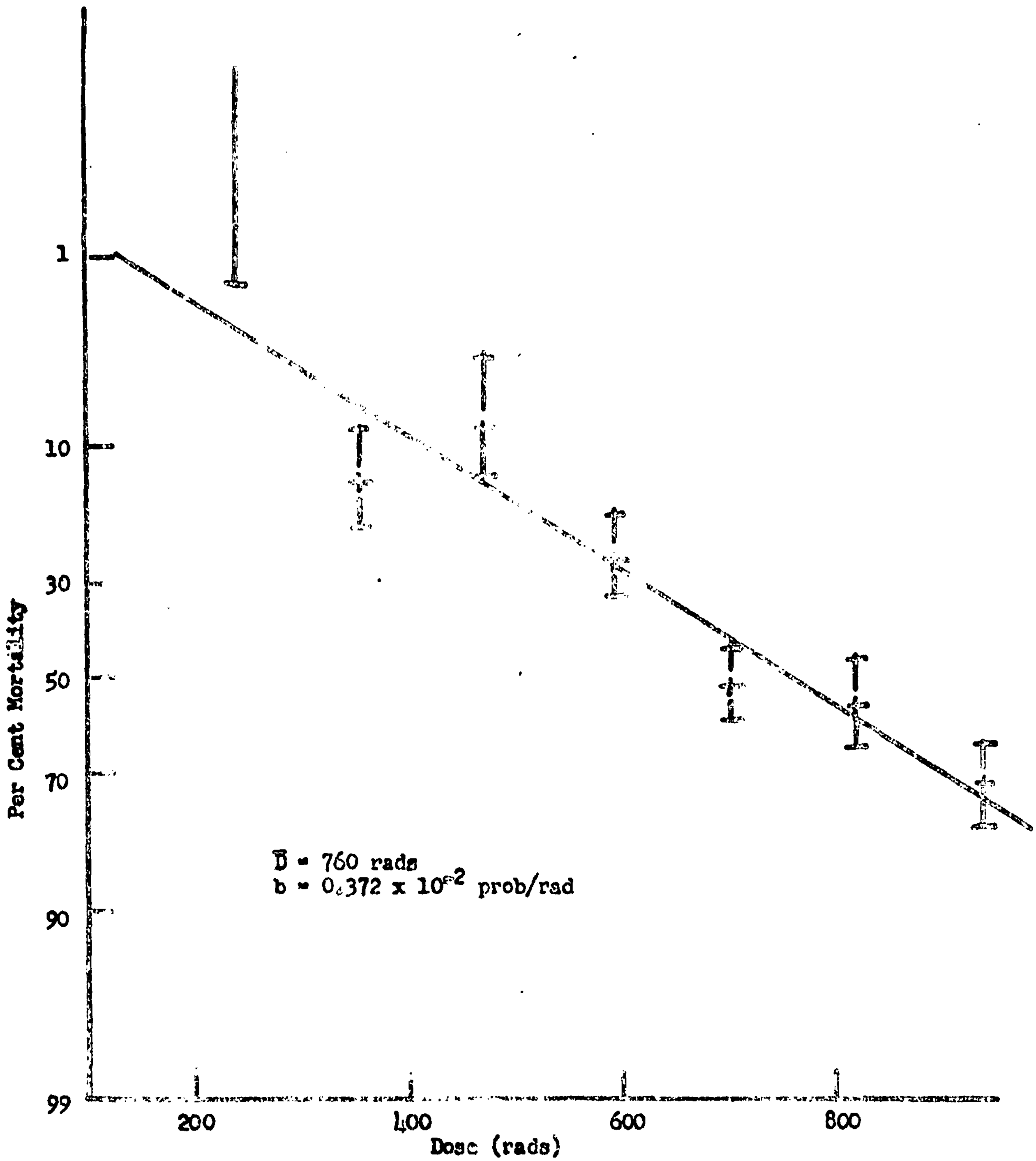


Figure 3. Dose-mortality data for CF_1 male mice exposed to whole-body proton irradiation.

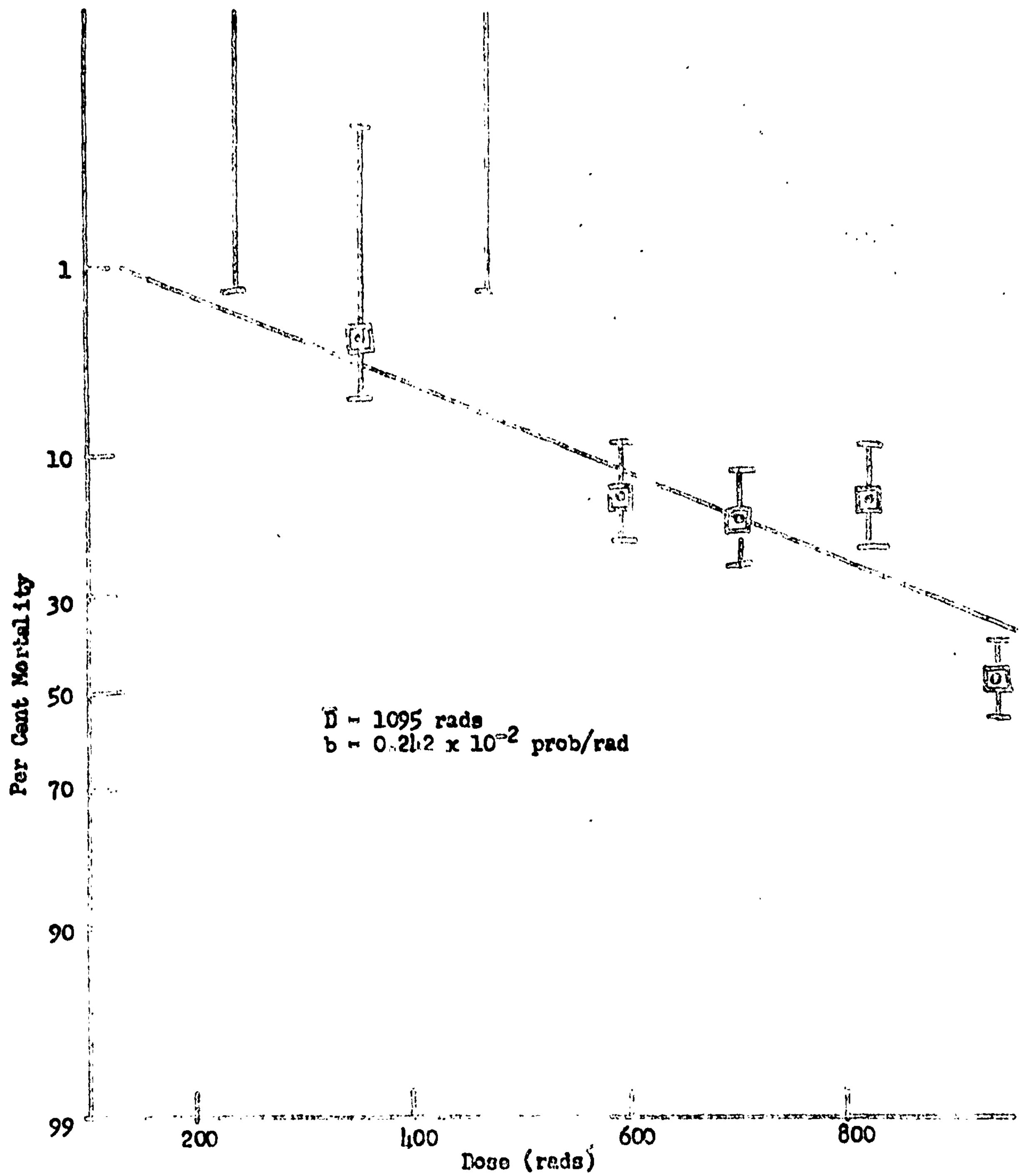


Figure 4. Dose-mortality data for CF_1 male mice exposed to whole-body proton irradiation following the administration of 30 mgm. PAPP/kgm.

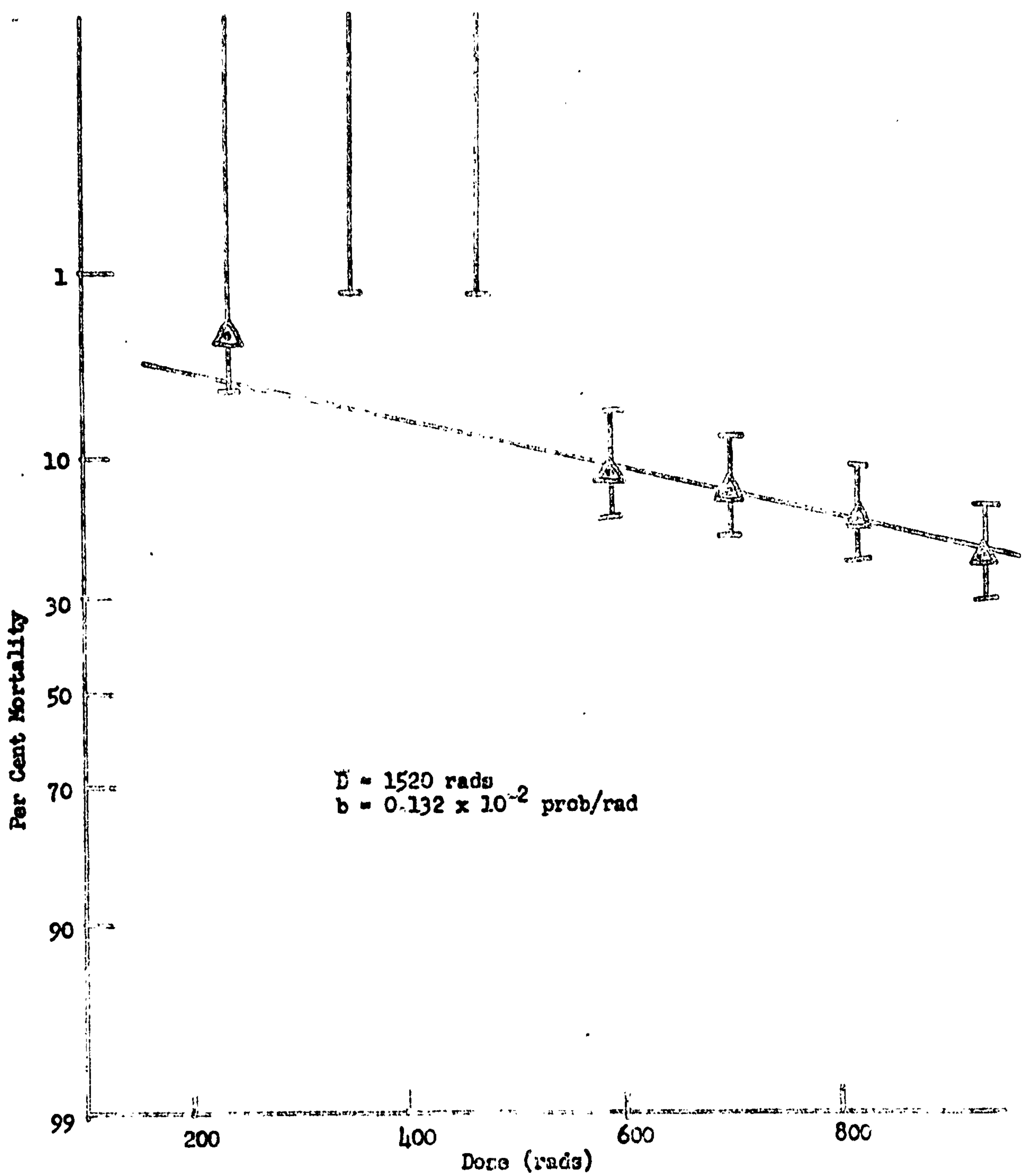


Figure 5. Dose-mortality data for CF_1 male mice exposed to whole-body proton irradiation following the administration of 225 mgm. MEA/kgm.

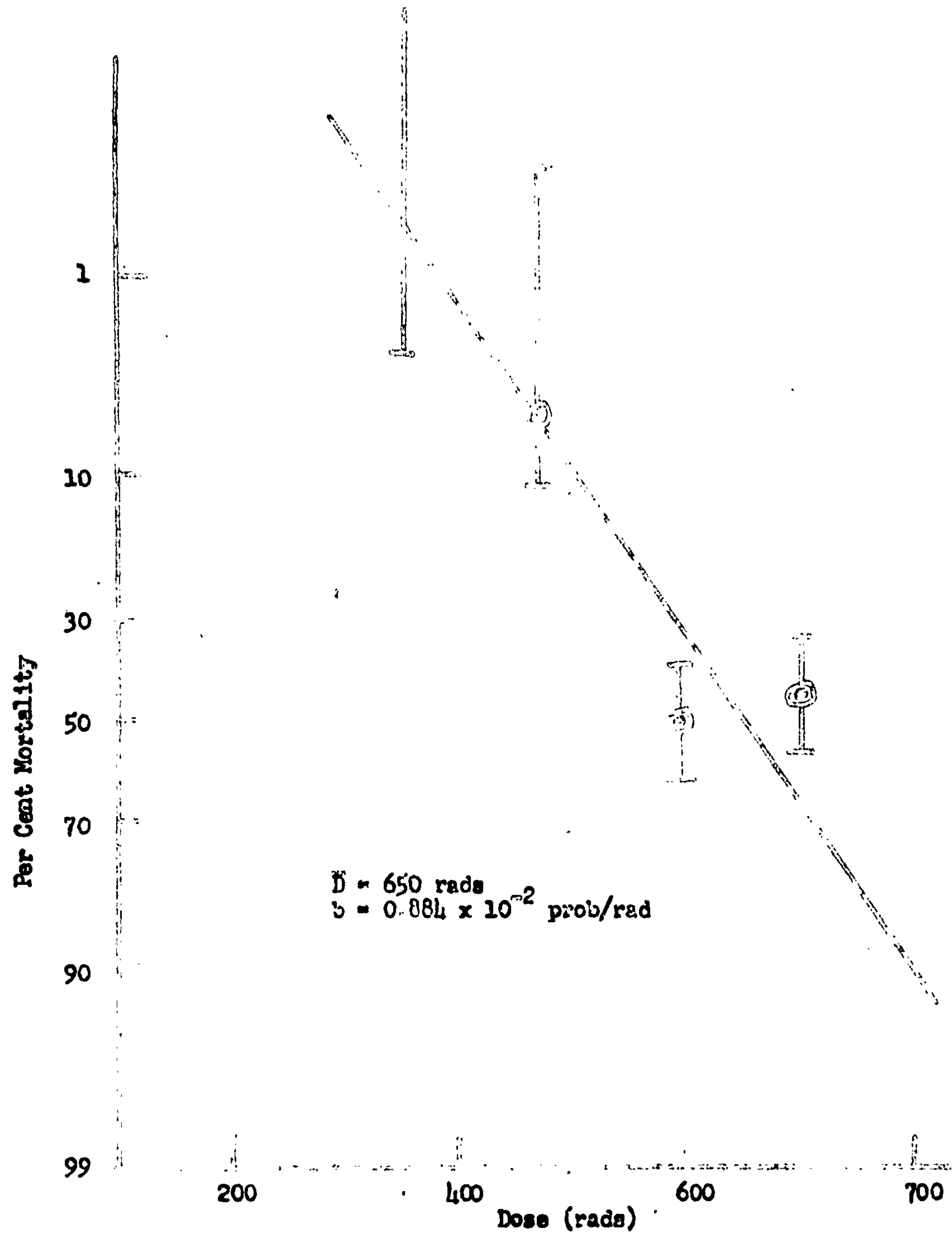


Figure 6. Dose-mortality data for CF_1 female mice exposed to whole-body proton irradiation.

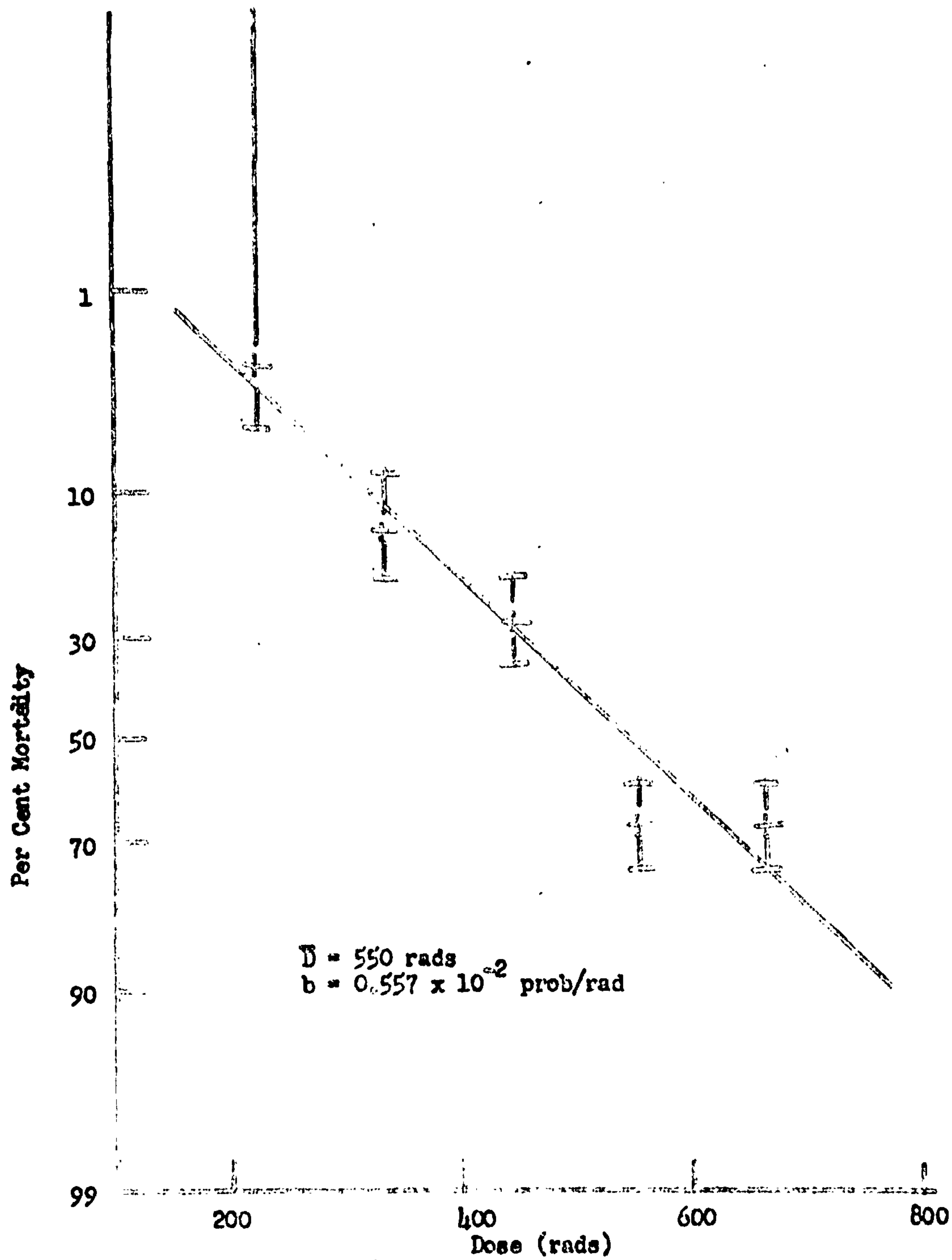


Figure 7. Dose-mortality data for CF_1 male mice exposed to whole body x irradiation.

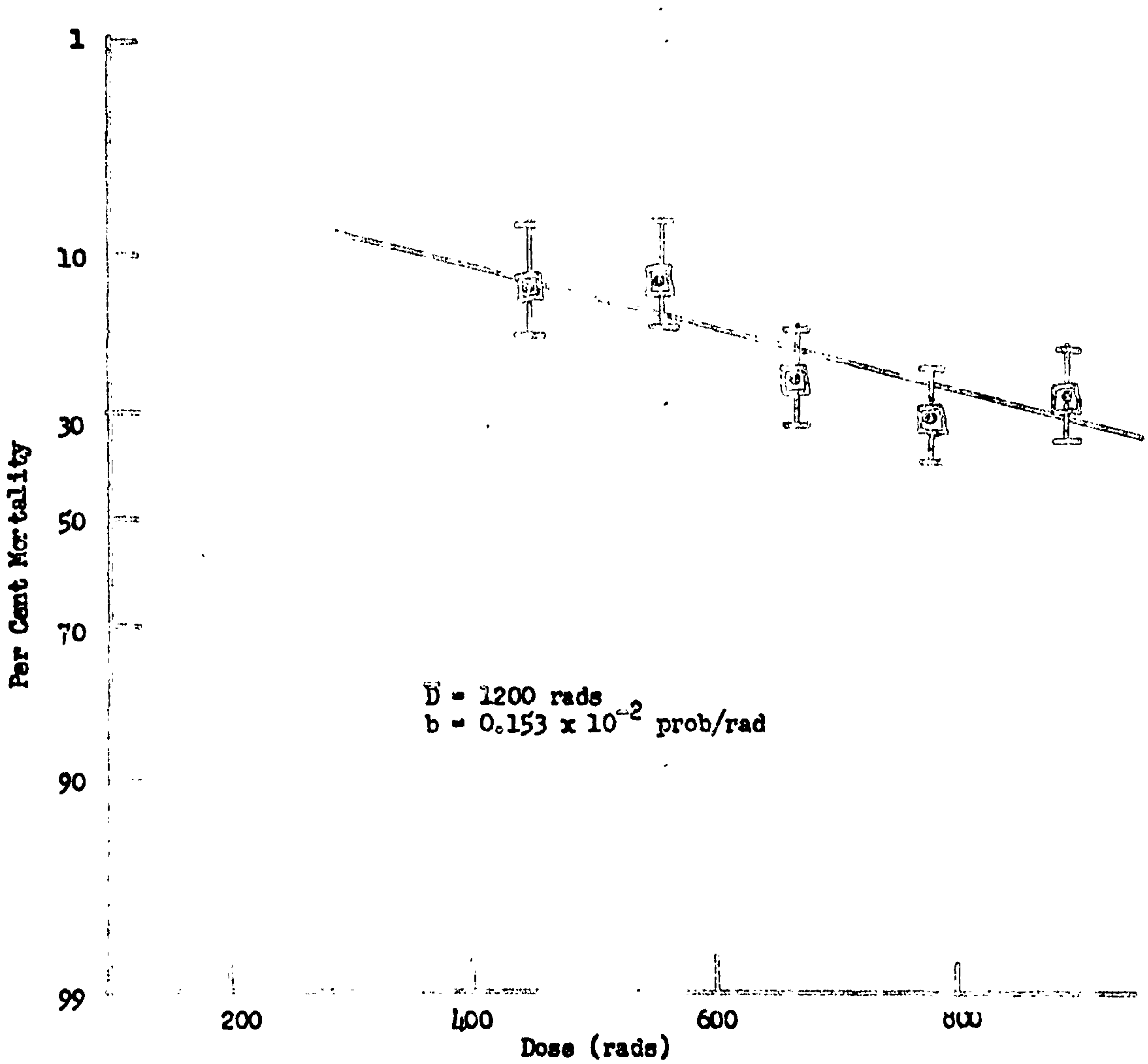


Figure 8. Dose-mortality data for CF_1 male mice exposed to whole-body x-irradiation following the administration of 30 mgm. PAPP/kgm.

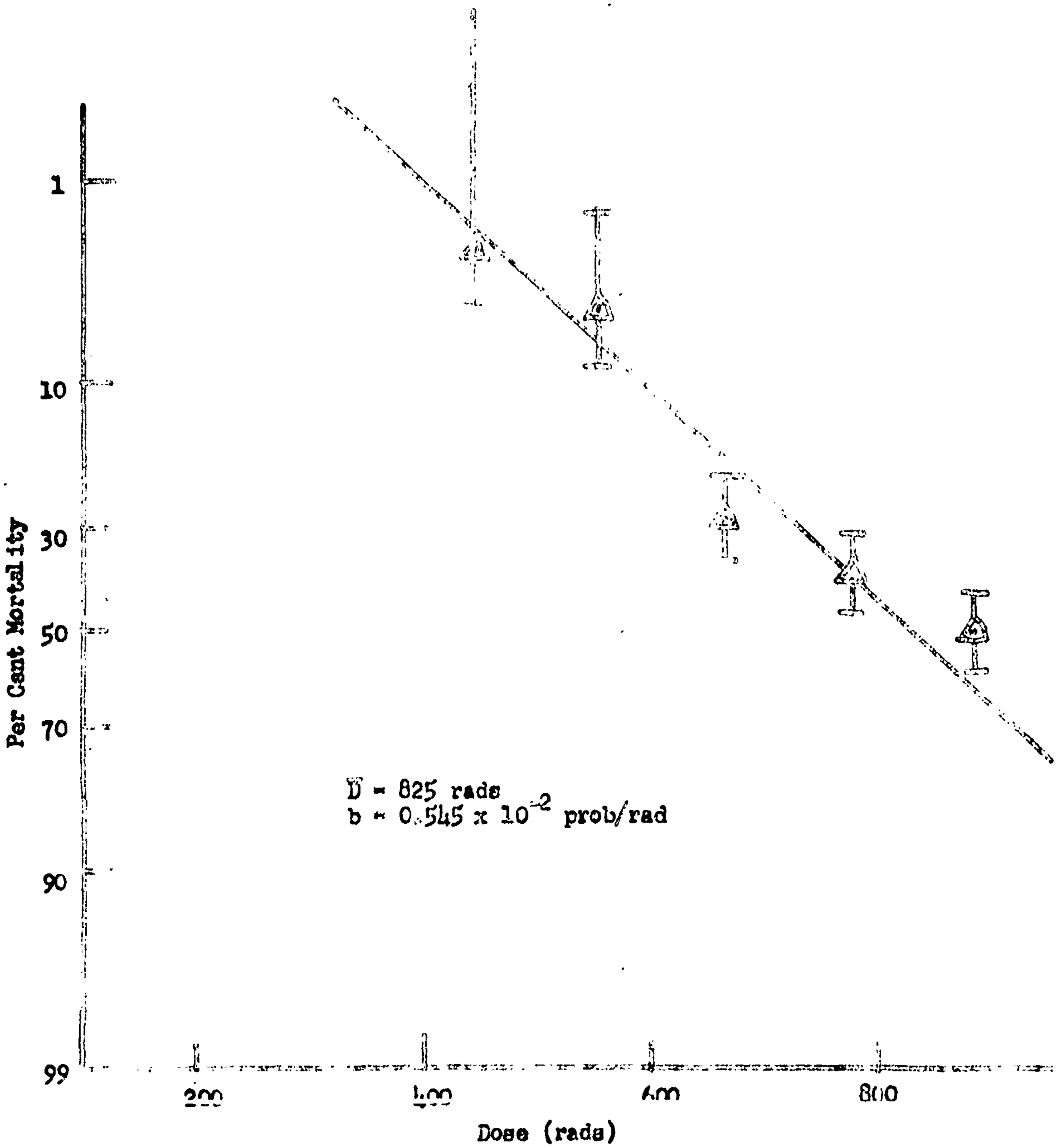


Figure 9. Dose-mortality data for CF_1 male mice exposed to whole-body x-irradiation following the administration of 225 mgm. MEA/kgm.

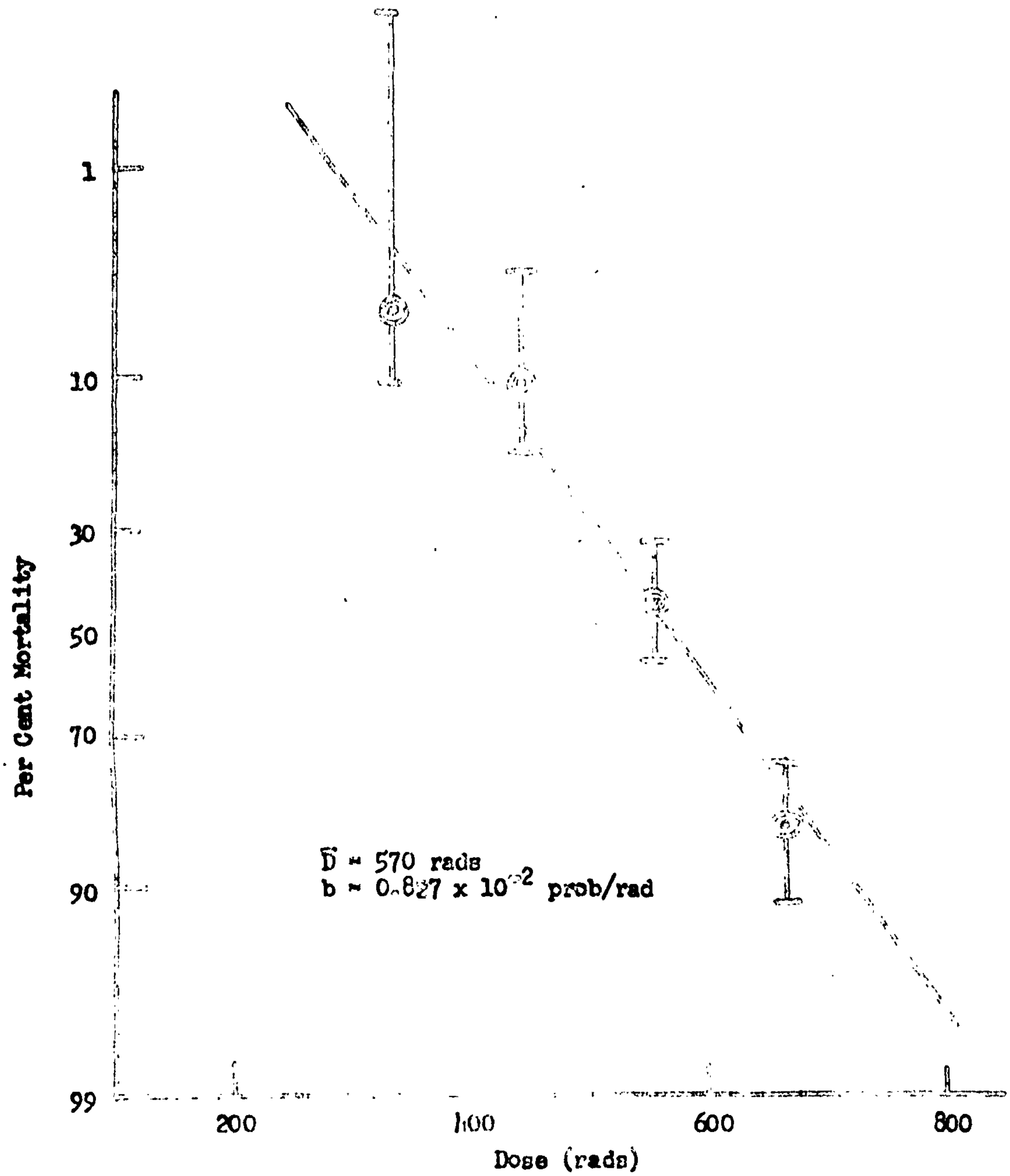


Figure 10. Dose-mortality data for CF_1 female mice exposed to whole-body x-irradiation.

TABLE 3

$LD_{50/30}$ ($\bar{D}$) AND REGRESSION LINE SLOPE (b) FOR PROTON AND X-RAY STUDIES

Group	$\bar{D}$ (rads)	b $\frac{\text{Probit}}{\text{Rad}}$	b·$\bar{D}$
Proton-control	760	0.372 x 10^{-2}	2.82
Proton-PAPP	1095	0.242	2.65
Proton-MEA	1520	0.132	2.01
Proton-control females	650	0.884	5.75
X-ray-control	550	0.557 x 10^{-2}	3.07
X-ray-PAPP	1200	0.153	1.83
X-ray-MEA	825	0.545	4.50
X-ray-control females	570	0.827	4.71

TABLE 4

DRF, RBE AND RPE VALUES FOR PROTON AND X RAY EXPOSURES

Radiation / Protector	Proton	X-ray	RBE = 0.72
PAPP	DRF = 1.44	DRF = 2.18	RPE = 0.91
MEA	DRF = 2.00	DRF = 1.50	RPE = 1.84

$$DRF = \frac{\bar{D}\text{-treated}}{\bar{D}\text{-control}} \qquad RPE = \frac{\bar{D}\ (x\text{-ray})}{\bar{D}\ (\text{proton})}$$

The mean dose, $\bar{D}$, for female controls is not appreciably different for either the males or between protons and x-rays. The slopes, b, however, are more steep than those for males for both radiations.

The distribution of deaths as a function of time within a group of mice that received a given dose and a given treatment also provides useful information for protection studies. These data are presented in hodograph form in Figure 11 for the 939 rad proton groups. Although the total number of mice involved is too small to permit a quantitative discussion, a number of qualitative observations can be made. First, in the control group, there appear to be two distinct periods during which the death rate exhibits peaks, one at about 6-8 days and the other at about 22 days. Second, a comparable pair of peaks is seen in the PAPP group, with some slight evidence of a shift in the peaks toward later times. Third, in the MEA group, only a single peak exists, and this peak seems also to be shifted to a slightly later time than the comparable first peak of the PAPP group. The implication of these results is that PAPP and MEA delay the onset of death in proton-irradiated mice and prolong the survival time.

Discussion

For the following discussion, we shall assume that the data on mortality is best represented as a linear relation between the mortality probit y and the absorbed radiation dose D. If future work indicates that the dependence of y is on some function of D (e.g., $\log D$) rather than D itself, it will be necessary to substitute that function wherever D appears now. Otherwise, the analysis should remain unchanged.

Suppose the mortality data satisfies the linear relation $y = 5 + b(D-\bar{D})$ where y is the probit corresponding to the mortality produced by the dose D. The dose $\bar{D}$ is, therefore, that which results in a probit $y = 5$, in other words, 50% mortality. The slope b is the rate at which the mortality probit increases with increasing dose. The two constants $\bar{D}$ and b completely characterize the response of the system.

There are at least three easily specified ways in which a chemical agent might alter radiation response as determined by cumulative mortality measured at 30 days. The simplest would be one in which the chemical agent did not alter the dose-response properties of the biological system itself, that is, the large molecules composing the system, but acted to decrease by a constant factor the absorbed dose effective in producing mortality by its action on large molecules. Such might be the case, for example, if the agent scavenged free radicals produced in water by the radiation. The regression equation for treated mice, $y' = 5 + b'(D-\bar{D}')$, should, therefore, be obtainable from that for untreated mice, $y = 5 + b(D-\bar{D})$, simply by substituting in the latter for D the value αD, where α is less than 1. We would then obtain $y' = 5 + b\alpha(D-\bar{D}/\alpha)$. Thus, both the slope, $b' = b\alpha$, and the 50% intercept, $\bar{D}' = \bar{D}/\alpha$, would be different when treated and untreated cases were compared. But more importantly, a specific relation would exist between the constants, namely,

$$b\bar{D} = b'\bar{D}'$$

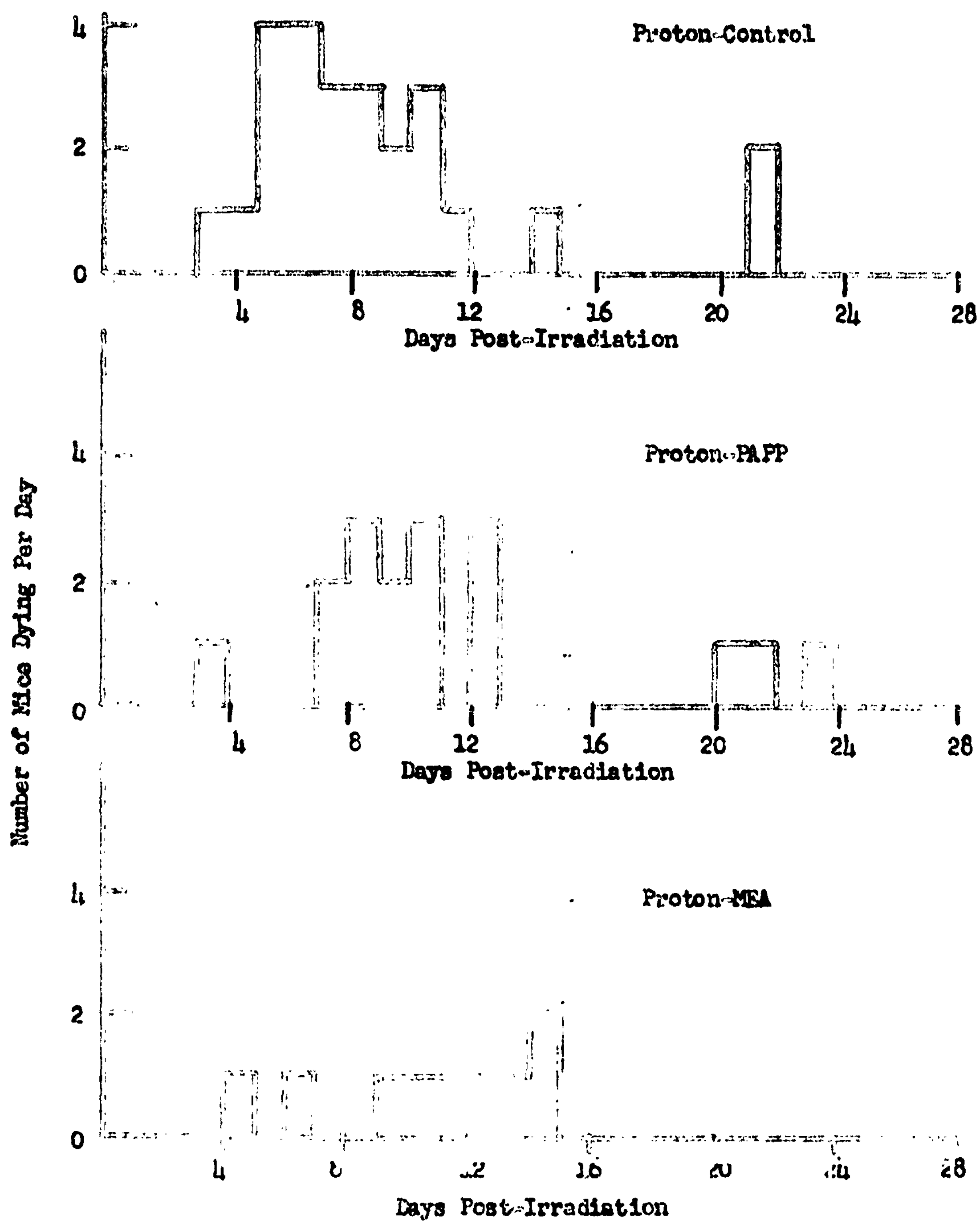


Figure 11. Death distribution in control and protected mice given 939 rads of proton irradiation.

If two types of radiation differ in their average LET, one may argue on the basis of an analysis similar to the above that the mortality curves produced by these radiations should also satisfy a relation of the type $b_1D_1 = b_2D_2$, the subscripts denoting the two different types of radiation.

The two other modes of altering radiation response would both involve an actual change in the dose-response parameters when the chemical agent was given. One would alter the rate, b, at which mortality probit changed with dose; the other would alter the absolute amount of dose required to bring the mortality probit to some selected value, say that corresponding to 50% mortality, $\bar{D}$. A change in the slope b clearly rotates the regression line about the dose $\bar{D}$; a change in $\bar{D}$ shifts the line parallel to itself. Of course, b and $\bar{D}$ might both change, but in this case there need not be any relation between the b and $\bar{D}$ values for treated and untreated groups.

Considering the $b \cdot \bar{D}$ values of Table 3, one sees that an approximate constancy of the product exists between proton-control and x-ray control groups, and also between proton-control and proton-PAPP groups. The relation of proton and x-ray curves is explicable on the basis of the arguments given earlier. Such a relation for the PAPP curve, and its absence for the MEA curve, suggest that the protection mechanism for PAPP at low levels of LET might involve a more purely physical mechanism than that for MEA; and that at higher levels of LET, neither substance acts in such purely physical fashion.

Summary

1. A method of proton and x-ray dosimetry using a Victoreen chamber charge-calibrated against a cobalt-60 gamma source producing a known field is described.

2. The radioprotective action of PAPP and MEA against 30-day lethality and rate of death in CF_1 mice exposed to whole-body 440 Mev protons is described and compared with that for 250 Kvp x-rays.

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